WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7: C12N 15/54, 9/10, 5/10, 15/89

(11) International Publication Number:

WO 00/09706

A2 (43) International Publication Date:

24 February 2000 (24.02.00)

(21) International Application Number:

PCT/US99/18760

(22) International Filing Date:

16 August 1999 (16.08.99)

(30) Priority Data:

60/096,822

17 August 1998 (17.08.98)

US

(71) Applicant (for all designated States except US): PIONEER HI-BRED INTERNATIONAL, INC. [US/US]; 800 Capital Square, 400 Locust Street, Des Moines, IA 50309 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): DHUGGA, Kanwarpal, S. [US/US]; 8320 Barnham Drive, Johnston, IA 50131 (US). HELENTIARIS, Timothy, G. [US/US]; 2960 N.W. 73rd Lane, Ankeny, IA 50021 (US). BOWEN, Benjamin, A. [GB/US]; 7027 Buckingham Boulevard, Berkeley, CA 94705 (US). WANG, Xun [CN/US]; 12524 Caminito Vista Soledad, San Diego, CA 92130 (US).

(74) Agents: BLAIR, Debra, L. et al.; 7100 N.W. 62nd Avenue, Darwin Building, Johnston, IA 50131-1000 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: MAIZE CELLULOSE SYNTHASES AND USES THEREOF

(57) Abstract

The invention provides isolated cellulose synthase nucleic acids and their encoded proteins. The present invention provides methods and compositions relating to altering cellulose synthase concentration and/or composition of plants. The invention further provides recombinant expression cassettes, host cells, and transgenic plants.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT

AL AM AT AU AZ BA BB BB BB BC BJ BR BY CA CCF CCG CCH CU CZ DE DK EE	Albania Armenia Austria Australia Azerbaijan Bosnia and Herzegovina Barbados Belgium Burkina Faso Bulgaria Benin Brazil Belarus Canada Central African Republic Congo Switzerland Côte d'Ivoire Cameroon China Cuba Czech Republic Germany Denmark Estonia	ES FI FR GA GB GCH GR HU IS IT JP KE KG KP LC LI LK LR	Spain Finland France Gabon United Kingdom Georgia Ghana Guinea Greece Hungary Ireland Israe! Iceland Israe! Iceland Isray Japan Kenya Kyngyzstan Democratic People's Republic of Korea Republic of Korea Kazakstan Saint Lucia Liechtenstein Sri Lanka Liberia	LS LT LU LV MC MD MG MK ML MN MN MR MW MX NE NL	Lesotho Lithuania Luxembourg Latvia Monaco Republic of Moldova Madagascar The former Yugoslav Republic of Macedonia Mali Mongolia Mauritania Malawi Mexico Niger Netherlands Norway New Zealand Poland Portugal Romania Russian Federation Sudan Sweden Singapore	SI SK SN SZ TD TG TJ TM TR TT UA UG US VN YU ZW	slovenia Slovenia Slovakia Senegal Swaziland Chad Togo Tajikistan Turkmenistan Turkey Trinidad and Tobago Ukraine Uganda United States of America Uzbekistan Viet Nam Yugoslavia Zimbabwe
--	--	--	--	---	---	---	---

Maize Cellulose Synthases and Uses Thereof

TECHNICAL FIELD

The present invention relates generally to plant molecular biology. More specifically, it relates to nucleic acids and methods for modulating their expression in plants.

BACKGROUND OF THE INVENTION

Polysaccharides constitute the bulk of the plant cell walls and have been traditionally classified into three categories: cellulose, hemicellulose, and pectin. Fry, S. C. (1988), The growing plant cell wall: Chemical and metabolic analysis. New York: Longman Scientific & Technical. Whereas cellulose is made at the plasma membrane and directly laid down into the cell wall, hemicellulosic and pectic polymers are first made in the Golgi apparatus and then exported to the cell wall by exocytosis. Ray, P.

M., et al., (1976), Ber. Deutsch. Bot. Ges. Bd. 89, 121-146. The variety of chemical linkages in the pectic and hemicellulosic polysaccharides indicates that there must be tens of polysaccharide synthases in the Golgi apparatus. Darvill et al., (1980). The primary cell walls of flowering plants. In The Plant Cell (N. E. Tolbert, ed.), Vol. 1 in Series: The biochemistry of plants: A comprehensive treatise, eds. P.K. Stumpf and E.E. Conn
(New York: Academic Press), pp. 91-162.

Cellulose, by virtue of its ability to form semicrystalline microfibrils, has a very high tensile strength which approaches that of some metals. Niklas, K. J. (1992). Plant Biomechanics: An engineering approach to plant form and function, The University of Chicago Press, pp. 607. Bending strength of the culm of normal and brittle-culm mutants of barley has been found to be directly correlated with the concentration of cellulose in the cell wall. Kokubo, et al., (1989), Plant Physiology 91, 876-882; Kokubo, et al., (1991) Plant Physiology 97, 509-514.

25

30

Even though sugar and polysaccharide compositions of the plant cell walls have been well characterized, very limited progress has been made toward identification of the enzymes involved in polysaccharides formation, the reason being their labile nature and recalcitrance to solubilization by available detergents. Sporadic claims for the identification of cellulose synthase from plant sources have been made over the years. Callaghan, T., and Benziman, M. (1984), Nature 311, 165-167; Okuda, et al., (1993),

- 2 -

Plant Physiol. 101, 1131-1142. However, these claims have been met with skepticism. Callaghan, T., and Benziman, M. (1985), *Nature* 314, 383-384; Delmer, *et al.*, (1993), Plant Physiol. 103, 307-308. It was only recently that a putative gene for plant cellulose synthase (CelA) was cloned from the developing cotton fibers based on homology to the bacterial gene. Pear, *et al.*, *Proc. Natl. Acad. Sci.* (USA) 93, 12637-12642; Saxena, *et al.*, (1990), *Plant Molecular Biology* 15, 673-684; see also, WO 9818949.

5

10

15

20

25

30

As brittle snap is a major problem in corn breeding, what is needed in the art are compositions and methods for manipulating cellulose concentration in the cell wall and thereby altering plant stalk quality for improved standability or silage. The present invention provides these and other advantages.

SUMMARY OF THE INVENTION

Generally, it is the object of the present invention to provide nucleic acids and proteins relating to cellulose synthases. It is an object of the present invention to provide: 1) nucleic acids and proteins relating to maize cellulose synthases; 2) transgenic plants comprising the nucleic acids of the present invention; 3) methods for modulating, in a transgenic plant, the expression of the nucleic acids of the present invention.

Therefore, in one aspect, the present invention relates to an isolated nucleic acid comprising a member selected from the group consisting of (a) a polynucleotide having a specified sequence identity to a polynucleotide encoding a polypeptide of the present invention;; (b) a polynucleotide which is complementary to the polynucleotide of (a); and (c) a polynucleotide comprising a specified number of contiguous nucleotides from a polynucleotide of (a) or (b). The isolated nucleic acid can be DNA or RNA.

In another aspect, the present invention relates to recombinant expression cassettes, comprising a nucleic acid of the present invention operably linked to a promoter. In some embodiments, the nucleic acid is operably linked in antisense orientation to the promoter.

In another aspect, the present invention is directed to a host cell transfected with the recombinant expression cassette.

In a further aspect, the present invention relates to an isolated protein comprising a polypeptide having a specified number of contiguous amino acids encoded by an isolated nucleic acid of the present invention.

- 3 -

In another aspect, the present invention relates to an isolated nucleic acid comprising a polynucleotide of specified length which selectively hybridizes under stringent conditions to a polynucleotide of the present invention, or a complement thereof. In some embodiments, the isolated nucleic acid is operably linked to a promoter.

5

10

15

20

25

30

In yet another aspect, the present invention relates to an isolated nucleic acid comprising a polynucleotide, the polynucleotide having a specified sequence identity to an identical length of a nucleic acid of the present invention or a complement thereof.

In another aspect, the present invention relates to an isolated nucleic acid comprising a polynucleotide having a sequence of a nucleic acid amplified from a Zea mays nucleic acid library using at least two primers or their complements, one of which selectively hyridizes under stringent conditions to a locus of the nucleic acid comprising the 5' terminal coding region and the other primer selectively hybridizing, under stringent conditions, to a locus of the nucleic acid comprising the 3' terminal coding region, and wherein both primers selectively hybridize within the coding region. In some embodiments, the nucleic acid library is a cDNA library.

In another aspect, the present invention relates to a recombinant expression cassette comprising a nucleic acid, wherein the nucleic acid is operably linked to a promoter. In some embodiments, the present invention relates to a host cell transfected with this recombinant expression cassette. In some embodiments, the present invention relates to a protein of the present invention which is produced from this host cell.

In a further aspect, the present invention relates to a heterologous promoter operably linked to a non-isolated polynucleotide of the present invention, wherein the polypeptide is encoded by a nucleic acid amplified from a nucleic acid library.

In yet another aspect, the present invention relates to a transgenic plant comprising a recombinant expression cassette comprising a plant promoter operably linked to any of the isolated nucleic acids of the present invention. In some embodiments, the transgenic plant is *Zea mays*. The present invention also provides transgenic seed from the transgenic plant.

In a further aspect, the present invention relates to a method of modulating expression of the genes encoding the proteins of the present invention in a plant cell capable of plant regeneration, comprising the steps of (a) transforming a plant cell with a recombinant expression cassette comprising a polynucleotide of the present invention

- 4 -

operably linked to a promoter; (b) growing the plant cell under plant growing conditions; and (c) inducing expression of the polynucleotide for a time sufficient to modulate expression of the genes in the plant. In some embodiments, the plant is maize. Expression of the genes encoding the proteins of the present invention can be increased or decreased relative to a non-transformed control plant.

Definitions

5

10

15

20

25

Units, prefixes, and symbols may be denoted in their SI accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range and include each integer within the defined range. Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. Unless otherwise provided for, software, electrical, and electronics terms as used herein are as defined in The New IEEE Standard Dictinary of Electrical and electronics Terms (5th edition, 1993). The terms defined below are more fully defined by reference to the specification as a whole.

By "amplified" is meant the construction of multiple copies of a nucleic acid sequence or multiple copies complementary to the nucleic acid sequence using at least one of the nucleic acid sequences as a template. Amplification systems include the polymerase chain reaction (PCR) system, ligase chain reaction (LCR) system, nucleic acid sequence based amplification (NASBA, Cangene, Mississauga, Ontario), Q-Beta Replicase systems, transcription-based amplification system (TAS), and strand displacement amplification (SDA). See, e.g., Diagnostic Molecular Microbiology. Principles and Applications, D. H. Persing et al., Ed., American Society for Microbiology, Washington, D.C. (1993). The product of amplification is termed an amplicon.

The term "antibody" includes reference to antigen binding forms of antibodies (e.g., Fab, F(ab)₂). The term "antibody" frequently refers to a polypeptide substantially encoded by an immunoglobulin gene or immunoglobulin genes, or fragments thereof which specifically bind and recognize an analyte (antigen). However, while various antibody fragments can be defined in terms of the digestion of an intact antibody, one of

- 5 -

skill will appreciate that such fragments may be synthesized *de novo* either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein, also includes antibody fragments such as single chain Fv, chimeric antibodies (i.e., comprising constant and variable regions from different species), humanized antibodies (i.e., comprising a complementarity determining region (CDR) from a non-human source) and heteroconjugate antibodies (e.g., bispecific antibodies).

5

10

15

20

25

30

The term "antigen" includes reference to a substance to which an antibody can be generated and/or to which the antibody is specifically immunoreactive. The specific immunoreactive sites within the antigen are known as epitopes or antigenic determinants. These epitopes can be a linear array of monomers in a polymeric composition - such as amino acids in a protein - or consist of or comprise a more complex secondary or tertiary structure. Those of skill will recognize that all immunogens (i.e., substances capable of eliciting an immune response) are antigens; however some antigens, such as haptens, are not immunogens but may be made immunogenic by coupling to a carrier molecule. An antibody immunologically reactive with a particular antigen can be generated *in vivo* or by recombinant methods such as selection of libraries of recombinant antibodies in phage or similar vectors. See, e.g., Huse et al., Science 246: 1275-1281 (1989); and Ward, et al., Nature 341: 544-546 (1989); and Vaughan et al., Nature Biotech. 14: 309-314 (1996).

As used herein, "antisense orientation" includes reference to a duplex polynucleotide sequence which is operably linked to a promoter in an orientation where the antisense strand is transcribed. The antisense strand is sufficiently complementary to an endogenous transcription product such that translation of the endogenous transcription product is often inhibited.

As used herein, "chromosomal region" includes reference to a length of a chromosome which may be measured by reference to the linear segment of DNA which it comprises. The chromosomal region can be defined by reference to two unique DNA sequences, i.e., markers.

The term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or conservatively modified variants of the amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein.

10

15

20

25

For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one species of conservatively modified variation. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine; and UGG, which is ordinarily the only codon for for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid which encodes a polypeptide of the present invention is implicit in each described polypeptide sequence and incorporated herein by reference.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically similar amino acid. Thus, any number of amino acid residues selected from the group of integers consisting of from 1 to 15 can be so altered. Thus, for example, 1, 2, 3, 4, 5, 7, or 10 alterations can be made. Conservatively modified variants typically provide similar biological activity as the unmodified polypeptide sequence from which they are derived. For example, substrate specificity, enzyme activity, or ligand/receptor binding is generally at least 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the native protein for it's native substrate. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 30 3) Asparagine (N), Glutamine (O);
 - 4) Arginine (R), Lysine (K);
 - 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
 - 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

-7 -

See also, Creighton (1984) Proteins W.H. Freeman and Company.

5

10

15

20

25

30

By "encoding" or "encoded", with respect to a specified nucleic acid, is meant comprising the information for translation into the specified protein. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid, or may lack such intervening non-translated sequences (e.g., as in cDNA). The information by which a protein is encoded is specified by the use of codons. Typically, the amino acid sequence is encoded by the nucleic acid using the "universal" genetic code. However, variants of the universal code, such as are present in some plant, animal, and fungal mitochondria, the bacterium Mycoplasma capricolum (Proc. Natl. Acad. Sci. (USA), 82: 2306-2309 (1985)), or the ciliate Macronucleus, may be used when the nucleic acid is expressed using these organisms.

When the nucleic acid is prepared or altered synthetically, advantage can be taken of known codon preferences of the intended host where the nucleic acid is to be expressed. For example, although nucleic acid sequences of the present invention may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ (Murray et al. Nucl. Acids Res. 17: 477-498 (1989)). Thus, the maize preferred codon for a particular amino acid may be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants are listed in Table 4 of Murray et al., above.

As used herein "full-length sequence" in reference to a specified polynucleotide or its encoded protein means having the entire amino acid sequence of, a native (non-synthetic), endogenous, catalytically active form of the specified protein. Methods to determine whether a sequence is full-length are well known in the art including such exemplary techniques as northern or western blots, primer extension, S1 protection, and ribonuclease protection. See, e.g., *Plant Molecular Biology: A Laboratory Manual*, Clark, Ed., Springer-Verlag, Berlin (1997). Comparison to known full-length homologous (orthologous and/or paralogous) sequences can also be used to identify full-length sequences of the present invention. Additionally, consensus sequences typically present at the 5' and 3' untranslated regions of mRNA aid in the identification of a polynucleotide as full-length. For example, the consensus sequence ANNNNAUGG,

WO 00/09706

5

10

15

20

25

30

where the underlined codon represents the N-terminal methionine, aids in determining whether the polynucleotide has a complete 5' end. Consensus sequences at the 3' end, such as polyadenylation sequences, aid in determining whether the polynucleotide has a complete 3' end.

The term "gene activity" refers to one or more steps involved in gene expression, including transcription, translation, and the functioning of the protein encoded by the gene.

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived, or, if from the same species, one or both are substantially modified from their original form. A heterologous protein may originate from a foreign species or, if from the same species, is substantially modified from its original form by deliberate human intervention.

By "host cell" is meant a cell which contains a vector and supports the replication and/or expression of the expression vector. Host cells may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, amphibian, or mammalian cells. Preferably, host cells are monocotyledonous or dicotyledonous plant cells. A particularly preferred monocotyledonous host cell is a maize host cell.

The term "hybridization complex" includes reference to a duplex nucleic acid structure formed by two single-stranded nucleic acid sequences selectively hybridized with each other.

By "immunologically reactive conditions" or "immunoreactive conditions" is meant conditions which allow an antibody, generated to a particular epitope, to bind to that epitope to a detectably greater degree (e.g., at least 2-fold over background) than the antibody binds to substantially all other epitopes in a reaction mixture comprising the particular epitope. Immunologically reactive conditions are dependent upon the format of the antibody binding reaction and typically are those utilized in immunoassay protocols. See Harlow and Lane, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York (1988), for a description of immunoassay formats and conditions.

-9-

The term "introduced" in the context of inserting a nucleic acid into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

The terms "isolated" refers to material, such as a nucleic acid or a protein, which is: (1) substantially or essentially free from components which normally accompany or interact with it as found in its naturally occurring environment. The isolated material optionally comprises material not found with the material in its natural environment; or (2) if the material is in its natural environment, the material has been synthetically (nonnaturally) altered by deliberate human intervention to a composition and/or placed at a locus in the cell (e.g., genome or subcellular organelle) not native to a material found in that environment. The alteration to yield the synthetic material can be performed on the material within or removed from its natural state. For example, a naturally occurring nucleic acid becomes an isolated nucleic acid if it is altered, or if it is transcribed from DNA which has been altered, by non-natural, synthetic (i.e., "man-made") methods performed within the cell from which it originates. See, e.g., Compounds and Methods for Site Directed Mutagenesis in Eukaryotic Cells, Kmiec, U.S. Patent No. 5,565,350; In Vivo Homologous Sequence Targeting in Eukaryotic Cells; Zarling et al., PCT/US93/03868. Likewise, a naturally occurring nucleic acid (e.g., a promoter) becomes isolated if it is introduced by non-naturally occurring means to a locus of the genome not native to that nucleic acid. Nucleic acids which are "isolated" as defined herein, are also referred to as "heterologous" nucleic acids.

10

15

20

25

30

Unless otherwise stated, the term "cellulose synthase nucleic acid" is a nucleic acid of the present invention and means a nucleic acid comprising a polynucleotide of the present invention (a "cellulose synthase polynucleotide") encoding a cellulose synthase polypeptide. A "cellulose synthase gene" is a gene of the present invention and refers to a non-heterologous genomic form of a full-length cellulose synthase polynucleotide.

As used herein, "localized within the chromosomal region defined by and including" with respect to particular markers includes reference to a contiguous length of a chromosome delimited by and including the stated markers.

- 10 -

As used herein, "marker" includes reference to a locus on a chromosome that serves to identify a unique position on the chromosome. A "polymorphic marker" includes reference to a marker which appears in multiple forms (alleles) such that different forms of the marker, when they are present in a homologous pair, allow transmission of each of the chromosomes in that pair to be followed. A genotype may be defined by use of one or a plurality of markers.

5

10

15

20

25

30

As used herein, "nucleic acid" includes reference to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues having the essential nature of natural nucleotides in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotides (e.g., peptide nucleic acids).

By "nucleic acid library" is meant a collection of isolated DNA or RNA molecules which comprise and substantially represent the entire transcribed fraction of a genome of a specified organism. Construction of exemplary nucleic acid libraries, such as genomic and cDNA libraries, is taught in standard molecular biology references such as Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology, Vol. 152, Academic Press, Inc., San Diego, CA (Berger); Sambrook et al., Molecular Cloning - A Laboratory Manual, 2nd ed., Vol. 1-3 (1989); and Current Protocols in Molecular Biology, F.M. Ausubel et al., Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (1994 Supplement).

As used herein "operably linked" includes reference to a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

As used herein, the term "plant" includes reference to whole plants, plant parts or organs (e.g., leaves, stems, roots, etc.), plant cells, seeds and progeny of same. Plant cell, as used herein includes, without limitation, cells obtained from or found in: seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. Plant cells can also be understood to include modified cells, such as protoplasts, obtained from the aforementioned tissues.

- 11 -

The class of plants which can be used in the methods of the invention is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants. Particularly preferred plants include maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley and millet.

5

10

15

20

25

30

As used herein, "polynucleotide" includes reference to a deoxyribopolynucleotide, ribopolynucleotide, or analogs thereof that have the essential nature of a natural ribonucleotide in that they hybridize, under stringent hybridization conditions, to substantially the same nucleotide sequence as naturally occurring nucleotides and/or allow translation into the same amino acid(s) as the naturally occurring nucleotide(s). A polynucleotide can be full-length or a subsequence of a native or heterologous structural or regulatory gene. Unless otherwise indicated, the term includes reference to the specified sequence as well as the complementary sequence thereof. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including among other things, simple and complex cells.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers. The essential nature of such analogues of naturally occurring amino acids is that, when incorporated into a protein, that protein is specifically reactive to antibodies elicited to the same protein but consisting entirely of naturally occurring amino acids. The terms "polypeptide", "peptide" and "protein" are also inclusive of modifications including, but not limited to, glycosylation, lipid attachment, sulfation, gamma-carboxylation of glutamic acid residues, hydroxylation and ADP-ribosylation. Exemplary modifications are described in most basic texts, such as, *Proteins - Structure*

and Molecular Properties, 2nd ed., T. E. Creighton, W. H. Freeman and Company, New York (1993). Many detailed reviews are available on this subject, such as, for example, those provided by Wold, F., Post-translational Protein Modifications: Perspectives and Prospects, pp. 1-12 in Posttranslational Covalent Modification of Proteins, B. C. Johnson, Ed., Academic Press, New York (1983); Seifter et al., Meth. Enzymol. 182: 626-646 (1990) and Rattan et al., Protein Synthesis: Posttranslational Modifications and Aging, Ann. N.Y. Acad. Sci. 663: 48-62 (1992). It will be appreciated, as is well known and as noted above, that polypeptides are not always entirely linear. For instance, polypeptides may be branched as a result of ubiquitination, and they may be circular, with or without branching, generally as a result of posttranslation events, including natural processing event and events brought about by human manipulation which do not occur naturally. Circular, branched and branched circular polypeptides may be synthesized by nontranslation natural process and by entirely synthetic methods, as well. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid sidechains and the amino or carboxyl termini. In fact, blockage of the amino or carboxyl group in a polypeptide, or both, by a covalent modification, is common in naturally occurring and synthetic polypeptides and such modifications may be present in polypeptides of the present invention, as well. For instance, the amino terminal residue of polypeptides made in E. coli or other cells, prior to proteolytic processing, almost invariably will be N-formylmethionine. During post-translational modification of the peptide, a methionine residue at the NH2-terminus may be deleted. Accordingly, this invention contemplates the use of both the methionine-containing and the methionine-less amino terminal variants of the protein of the invention. In general, as used herein, the term polypeptide encompasses all such modifications, particularly those that are present in polypeptides synthesized by expressing a polynucleotide in a host cell.

10

15

20

25

30

As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. Exemplary plant promoters include, but are not limited to, those that are obtained from plants, plant viruses, and bacteria which comprise genes expressed in plant cells such Agrobacterium or Rhizobium. Examples of promoters under developmental control include promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, or seeds. Such promoters are

WO 00/09706

10

15

20

25

30

PCT/US99/18760

referred to as "tissue preferred". Promoters which initiate transcription only in certain tissue are referred to as "tissue specific". A "cell type" specific promoter primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves. An "inducible" promoter is a promoter which is under environmental control. Examples of environmental conditions that may effect transcription by inducible promoters include anaerobic conditions or the presence of light. Tissue specific, tissue preferred, cell type specific, and inducible promoters constitute the class of "non-constitutive" promoters. A "constitutive" promoter is a promoter which is active under most environmental conditions.

- 13 -

The term "cellulose synthase polypeptide" is a polypeptide of the present invention and refers to one or more amino acid sequences, in glycosylated or non-glycosylated form. The term is also inclusive of fragments, variants, homologs, alleles or precursors (e.g., preproproteins or proproteins) thereof. A "cellulose synthase protein" is a protein of the present invention and comprises a cellulose synthase polypeptide.

As used herein "recombinant" includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found in identical form within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under-expressed or not expressed at all as a result of deliberate human intervention. The term "recombinant" as used herein does not encompass the alteration of the cell or vector by naturally occurring events (e.g., spontaneous mutation, natural transformation/transduction/transposition) such as those occurring without deliberate human intervention.

As used herein, a "recombinant expression cassette" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements which permit transcription of a particular nucleic acid in a host cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid to be transcribed, and a promoter.

The term "residue" or "amino acid residue" or "amino acid" are used interchangeably herein to refer to an amino acid that is incorporated into a protein,

- 14 -

polypeptide, or peptide (collectively "protein"). The amino acid may be a naturally occurring amino acid and, unless otherwise limited, may encompass known analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

5

10

15

20

25

30

The term "selectively hybridizes" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing sequences typically have about at least 80% sequence identity, preferably 90% sequence identity, and most preferably 100% sequence identity (i.e., complementary) with each other.

The term "specifically reactive", includes reference to a binding reaction between an antibody and a protein having an epitope recognized by the antigen binding site of the antibody. This binding reaction is determinative of the presence of a protein having the recognized epitope amongst the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to an analyte having the recognized epitope to a substantially greater degree (e.g., at least 2-fold over background) than to substantially all other analytes lacking the epitope which are present in the sample.

The terms "stringent conditions" or "stringent hybridization conditions" includes reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified which are 100% complementary to the probe (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50

nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C.

10 Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, Anal. Biochem., 138:267-284 (1984): $T_m = 81.5 \text{ °C} + 16.6 (\log M) + 0.41 (\% GC) - 10.00 (\log M) + 0.41 (\% GC)$ 0.61 (% form) - 500/L; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage 15 of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. $T_{\rm m}$ is reduced by about 1 °C for each 1% of mismatching; thus, T_m, hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For 20 example, if sequences with $\geq 90\%$ identity are sought, the T_m can be decreased 10 °C. Generally, stringent conditions are selected to be about 5 °C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization 25 and/or wash at 1, 2, 3, or 4 °C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10 °C lower than the thermal melting point (T_m) ; low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20 °C lower than the thermal melting point (T_m) . Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or 30 wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45 °C (aqueous solution) or 32 °C (formamide solution) it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive

10

15

20

25

30

guide to the hybridization of nucleic acids is found in Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes, Part I, Chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York (1993); and Current Protocols in Molecular Biology, Chapter 2, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995).

As used herein, "transgenic plant" includes reference to a plant which comprises within its genome a heterologous polynucleotide. Generally, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is used herein to include any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been altered by the presence of heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

As used herein, "vector" includes reference to a nucleic acid used in transfection of a host cell and into which can be inserted a polynucleotide. Vectors are often replicons. Expression vectors permit transcription of a nucleic acid inserted therein.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", (d) "percentage of sequence identity", and (e) "substantial identity".

- (a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.
- (b) As used herein, "comparison window" means includes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence may be compared to a reference sequence and wherein the

portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of sequences for comparison are well-known in the art. 10 Optimal alignment of sequences for comparison may be conducted by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2: 482 (1981); by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48: 443 (1970); by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. 85: 2444 (1988); by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California, 15 GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wisconsin, USA; the CLUSTAL program is well described by Higgins and Sharp, Gene 73: 237-244 (1988); Higgins and Sharp, CABIOS 5: 151-153 (1989); Corpet, et al., Nucleic Acids Research 16: 10881-90 (1988); Huang, et al., Computer Applications in the 20 Biosciences 8: 155-65 (1992), and Pearson, et al., Methods in Molecular Biology 24: 307-331 (1994). The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; 25 TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, Current Protocols in Molecular Biology, Chapter 19, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995). 30

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using the BLAST 2.0 suite of programs using default parameters.

Altschul et al., Nucleic Acids Res. 25:3389-3402 (1997). Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology

WO 00/09706

5

10

15

20

25

30

Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915).

In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance.

BLAST searches assume that proteins can be modeled as random sequences. However, many real proteins comprise regions of nonrandom sequences which may be homopolymeric tracts, short-period repeats, or regions enriched in one or more amino acids. Such low-complexity regions may be aligned between unrelated proteins even though other regions of the protein are entirely dissimilar. A number of low-complexity filter programs can be employed to reduce such low-complexity alignments. For example, the SEG (Wooten and Federhen, *Comput. Chem.*, 17:149-163 (1993)) and

- 19 -

XNU (Claverie and States, *Comput. Chem.*, 17:191-201 (1993)) low-complexity filters can be employed alone or in combination.

5

10

15

20

25

30

- (c) As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g. charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences which differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well-known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., according to the algorithm of Meyers and Miller, Computer Applic. Biol. Sci., 4: 11-17 (1988) e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California, USA).
- (d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

10

15

20

25

30

(e) (i) The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has at least 70% sequence identity, preferably at least 80%, more preferably at least 90% and most preferably at least 95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of at least 60%, more preferably at least 70%, 80%, 90%, and most preferably at least 95%.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. However, nucleic acids which do not hybridize to each other under stringent conditions are still substantially identical if the polypeptides which they encode are substantially identical. This may occur, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is that the polypeptide which the first nucleic acid encodes is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

(e) (ii) The terms "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with at least 70% sequence identity to a reference sequence, preferably 80%, more preferably 85%, most preferably at least 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48: 443 (1970). An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. Peptides which are "substantially similar" share sequences as noted above except that residue positions which are not identical may differ by conservative amino acid changes.

- 21 -

Overview

5

10

15

20

25

30

The present invention provides, among other things, compositions and methods for modulating (i.e., increasing or decreasing) the level of polypeptides of the present invention in plants. In particular, the polypeptides of the present invention can be expressed at developmental stages, in tissues, and/or in quantities which are uncharacteristic of non-recombinantly engineered plants. Thus, the present invention provides utility in such exemplary applications as improvement of stalk quality for improved stand or silage. Further, the present invention provides for an increased concentration of cellulose in the pericarp; hardening the kernel and thus improving its handling ability.

The present invention also provides isolated nucleic acid comprising polynucleotides of sufficient length and complementarity to a gene of the present invention to use as probes or amplification primers in the detection, quantitation, or isolation of gene transcripts. For example, isolated nucleic acids of the present invention can be used as probes in detecting deficiencies in the level of mRNA in screenings for desired transgenic plants, for detecting mutations in the gene (e.g., substitutions, deletions, or additions), for monitoring upregulation of expression or changes in enzyme activity in screening assays of compounds, for detection of any number of allelic variants (polymorphisms) of the gene, or for use as molecular markers in plant breeding programs. The isolated nucleic acids of the present invention can also be used for recombinant expression of their encoded polypeptides, or for use as immunogens in the preparation and/or screening of antibodies. The isolated nucleic acids of the present invention can also be employed for use in sense or antisense suppression of one or more genes of the present invention in a host cell, tissue, or plant. Attachment of chemical agents which bind, intercalate, cleave and/or crosslink to the isolated nucleic acids of the present invention can also be used to modulate transcription or translation.

The present invention also provides isolated proteins comprising a polypeptide of the present invention (e.g., preproenzyme, proenzyme, or enzymes). The present invention also provides proteins comprising at least one epitope from a polypeptide of the present invention. The proteins of the present invention can be employed in assays for enzyme agonists or antagonists of enzyme function, or for use as immunogens or antigens to obtain antibodies specifically immunoreactive with a protein of the present invention. Such antibodies can be used in assays for expression levels, for identifying

and/or isolating nucleic acids of the present invention from expression libraries, or for purification of polypeptides of the present invention.

The isolated nucleic acids and proteins of the present invention can be used over a broad range of plant types, particularly monocots such as the species of the Family

5 Graminiae including Sorghum bicolor and Zea mays. The isolated nucleic acid and proteins of the present invention can also be used in species from the genera: Cucurbita, Rosa, Vitis, Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solanum,

10 Petunia, Digitalis, Majorana, Ciahorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Avena, Hordeum, Secale, Triticum, Bambusa, Dendrocalamus, and Melocanna.

15 Nucleic Acids

The present invention provides, *among other things*, isolated nucleic acids of RNA, DNA, and analogs and/or chimeras thereof, comprising a polynucleotide of the present invention.

A polynucleotide of the present invention is inclusive of:

- 20 (a) a polynucleotide encoding a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58, and conservatively modified and polymorphic variants thereof, including exemplary polynucleotides of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57;
- (b) a polynucleotide which is the product of amplification from a Zea mays

 nucleic acid library using primer pairs which selectively hybridize under stringent
 conditions to loci within a polynucleotide selected from the group consisting of SEQ ID

 NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57, wherein the
 polynucleotide has substantial sequence identity to a polynucleotide selected from the
 group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53,

 and 57;
 - (c) a polynucleotide which selectively hybridizes to a polynucleotide of (a) or (b);
 - (d) a polynucleotide having a specified sequence identity with polynucleotides of (a), (b), or (c);

- (e) a polynucleotide encoding a protein having a specified number of contiguous amino acids from a prototype polypeptide, wherein the protein is specifically recognized by antisera elicited by presentation of the protein and wherein the protein does not detectably immunoreact to antisera which has been fully immunosorbed with the protein;
 - (f) complementary sequences of polynucleotides of (a), (b), (c), (d), or (e); and
- (g) a polynucleotide comprising at least a specific number of contiguous nucleotides from a polynucleotide of (a), (b), (c), (d), (e), or (f).
- A. Polynucleotides Encoding A Polypeptide of the Present Invention or Conservatively

 Modified or Polymorphic Variants Thereof

As indicated in (a), above, the present invention provides isolated nucleic acids comprising a polynucleotide of the present invention, wherein the polynucleotide encodes a polypeptide of the present invention, or conservatively modified or polymorphic variants thereof. Those of skill in the art will recognize that the degeneracy of the 15 genetic code allows for a plurality of polynucleotides to encode for the identical amino acid sequence. Such "silent variations" can be used, for example, to selectively hybridize and detect allelic variants of polynucleotides of the present invention. Accordingly, the present invention includes polynucleotides of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57, and silent variations of 20 polynucleotides encoding a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58. The present invention further provides isolated nucleic acids comprising polynucleotides encoding conservatively modified variants of a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58. Additionally, the present invention further provides isolated nucleic acids comprising 25 polynucleotides encoding one or more polymorphic (allelic) variants of polypeptides/polynucleotides. Polymorphic variants are frequently used to follow segregation of chromosomal regions in, for example, marker assisted selection methods for crop improvement.

5

10

15

25

30

As indicated in (b), above, the present invention provides an isolated nucleic acid comprising a polynucleotide of the present invention, wherein the polynucleotides are amplified from a Zea mays nucleic acid library. Zea mays lines B73, PHRE1, A632, BMS-P2#10, W23, and Mo17 are known and publicly available. Other publicly known and available maize lines can be obtained from the Maize Genetics Cooperation (Urbana, IL). The nucleic acid library may be a cDNA library, a genomic library, or a library generally constructed from nuclear transcripts at any stage of intron processing. cDNA libraries can be normalized to increase the representation of relatively rare cDNAs. In optional embodiments, the cDNA library is constructed using a full-length cDNA synthesis method. Examples of such methods include Oligo-Capping (Maruyama, K. and Sugano, S. Gene 138: 171-174, 1994), Biotinylated CAP Trapper (Carninci, P., Kvan, C., et al. Genomics 37: 327-336, 1996), and CAP Retention Procedure (Edery, E., Chu, L.L., et al. Molecular and Cellular Biology 15: 3363-3371, 1995). cDNA synthesis is often catalyzed at 50-55°C to prevent formation of RNA secondary structure. Examples of reverse transcriptases that are relatively stable at these temperatures are SuperScript II Reverse Transcriptase (Life Technologies, Inc.), AMV Reverse Transcriptase (Boehringer Mannheim) and RetroAmp Reverse Transcriptase (Epicentre). Rapidly growing tissues, or rapidly dividing cells are preferably used as mRNA sources such as from the elongating internode of corn plants.

The polynucleotides of the present invention include those amplified using the following primer pairs:

SEQ ID NOS: 3 and 4 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 1;

SEQ ID NOS: 7 and 8 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 5; and

SEQ ID NOS: 11 and 12 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 9.

SEQ ID NOS: 15 and 16 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 13.

SEQ ID NOS: 19 and 20 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 17;

10

15

20

25

30

SEQ ID NOS: 23 and 24 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 21; and

SEQ ID NOS: 27 and 28 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 25.

SEQ ID NOS: 31 and 32 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 29.

SEQ ID NOS: 35 and 36 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 33;

SEQ ID NOS: 39 and 40 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 37; and

SEQ ID NOS: 43 and 44 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 41.

SEQ ID NOS: 47 and 48 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 45.

SEQ ID NOS: 51 and 52 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 49;

SEQ ID NOS: 55 and 56 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 53; and

SEQ ID NOS: 59 and 60 which yield an amplicon comprising a sequence having substantial identity to SEQ ID NO: 57.

The present invention also provides subsequences of the polynucleotides of the present invention. A variety of subsequences can be obtained using primers which selectively hybridize under stringent conditions to at least two sites within a polynucleotide of the present invention, or to two sites within the nucleic acid which flank and comprise a polynucleotide of the present invention, or to a site within a polynucleotide of the present invention and a site within the nucleic acid which comprises it. Primers are chosen to selectively hybridize, under stringent hybridization conditions, to a polynucleotide of the present invention. Generally, the primers are complementary to a subsequence of the target nucleic acid which they amplify. As those skilled in the art will appreciate, the sites to which the primer pairs will selectively hybridize are chosen such that a single contiguous nucleic acid can be formed under the desired amplification conditions.

- 26 -

In optional embodiments, the primers will be constructed so that they selectively hybridize under stringent conditions to a sequence (or its complement) within the target nucleic acid which comprises the codon encoding the carboxy or amino terminal amino acid residue (i.e., the 3' terminal coding region and 5' terminal coding region,

respectively) of the polynucleotides of the present invention. Optionally within these embodiments, the primers will be constructed to selectively hybridize entirely within the coding region of the target polynucleotide of the present invention such that the product of amplification of a cDNA target will consist of the coding region of that cDNA. The primer length in nucleotides is selected from the group of integers consisting of from at least 15 to 50. Thus, the primers can be at least 15, 18, 20, 25, 30, 40, or 50 nucleotides in length. Those of skill will recognize that a lengthened primer sequence can be employed to increase specificity of binding (i.e., annealing) to a target sequence. A non-annealing sequence at the 5'end of a primer (a "tail") can be added, for example, to introduce a cloning site at the terminal ends of the amplicon.

The amplification products can be translated using expression systems well known to those of skill in the art and as discussed, *infra*. The resulting translation products can be confirmed as polypeptides of the present invention by, for example, assaying for the appropriate catalytic activity (e.g., specific activity and/or substrate specificity), or verifying the presence of one or more linear epitopes which are specific to a polypeptide of the present invention. Methods for protein synthesis from PCR derived templates are known in the art and available commercially. See, e.g., Amersham Life Sciences, Inc, Catalog '97, p.354.

Methods for obtaining 5' and/or 3' ends of a vector insert are well known in the art. See, e.g., RACE (Rapid Amplification of Complementary Ends) as described in Frohman, M. A., in PCR Protocols: A Guide to Methods and Applications, M. A. Innis, D. H. Gelfand, J. J. Sninsky, T. J. White, Eds. (Academic Press, Inc., San Diego, 1990), pp. 28-38.); see also, U.S. Pat. No. 5,470,722, and *Current Protocols in Molecular Biology*, Unit 15.6, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995); Frohman and Martin, *Techniques* 1:165 (1989).

30

5

10

15

20

25

C. Polynucleotides Which Selectively Hybridize to a Polynucleotide of (A) or (B)

As indicated in (c), above, the present invention provides isolated nucleic acids comprising polynucleotides of the present invention, wherein the polynucleotides

10

15

25

selectively hybridize, under selective hybridization conditions, to a polynucleotide of paragraphs (A) or (B) as discussed, above. Thus, the polynucleotides of this embodiment can be used for isolating, detecting, and/or quantifying nucleic acids comprising the polynucleotides of (A) or (B). For example, polynucleotides of the present invention can be used to identify, isolate, or amplify partial or full-length clones in a deposited library. In some embodiments, the polynucleotides are genomic or cDNA sequences isolated or otherwise complementary to a cDNA from a dicot or monocot nucleic acid library. Exemplary species of monocots and dicots include, but are not limited to: corn, canola, soybean, cotton, wheat, sorghum, sunflower, oats, sugar cane, millet, barley, and rice. Optionally, the cDNA library comprises at least 80% full-length sequences, preferably at least 85% or 90% full-length sequences, and more preferably at least 95% full-length sequences. The cDNA libraries can be normalized to increase the representation of rare sequences. Low stringency hybridization conditions are typically, but not exclusively, employed with sequences having a reduced sequence identity relative to complementary sequences. Moderate and high stringency conditions can optionally be employed for sequences of greater identity. Low stringency conditions allow selective hybridization of sequences having about 70% sequence identity and can be employed to identify orthologous or paralogous sequences.

D. Polynucleotides Having a Specific Sequence Identity with the Polynucleotides of (A), (B) or (C)

As indicated in (d), above, the present invention provides isolated nucleic acids comprising polynucleotides of the present invention, wherein the polynucleotides have a specified identity at the nucleotide level to a polynucleotide as disclosed above in paragraphs (A), (B), or (C). The percentage of identity to a reference sequence is at least 60% and, rounded upwards to the nearest integer, can be expressed as an integer selected from the group of integers consisting of from 60 to 99. Thus, for example, the percentage of identity to a reference sequence can be at least 70%, 75%, 80%, 85%, 90%, or 95%.

Optionally, the polynucleotides of this embodiment will share an epitope with a polypeptide encoded by the polynucleotides of (A), (B), or (C). Thus, these polynucleotides encode a first polypeptide which elicits production of antisera comprising antibodies which are specifically reactive to a second polypeptide encoded by

10

15

20

25

30

a polynucleotide of (A), (B), or (C). However, the first polypeptide does not bind to antisera raised against itself when the antisera has been fully immunosorbed with the first polypeptide. Hence, the polynucleotides of this embodiment can be used to generate antibodies for use in, for example, the screening of expression libraries for nucleic acids comprising polynucleotides of (A), (B), or (C), or for purification of, or in immunoassays for, polypeptides encoded by the polynucleotides of (A), (B), or (C). The polynucleotides of this embodiment embrace nucleic acid sequences which can be employed for selective hybridization to a polynucleotide encoding a polypeptide of the present invention.

Screening polypeptides for specific binding to antisera can be conveniently achieved using peptide display libraries. This method involves the screening of large collections of peptides for individual members having the desired function or structure. Antibody screening of peptide display libraries is well known in the art. The displayed peptide sequences can be from 3 to 5000 or more amino acids in length, frequently from 5-100 amino acids long, and often from about 8 to 15 amino acids long. In addition to direct chemical synthetic methods for generating peptide libraries, several recombinant DNA methods have been described. One type involves the display of a peptide sequence on the surface of a bacteriophage or cell. Each bacteriophage or cell contains the nucleotide sequence encoding the particular displayed peptide sequence. Such methods are described in PCT patent publication Nos. 91/17271, 91/18980, 91/19818, and 93/08278. Other systems for generating libraries of peptides have aspects of both in vitro chemical synthesis and recombinant methods. See, PCT Patent publication Nos. 92/05258, 92/14843, and 96/19256. See also, U.S. Patent Nos. 5,658,754; and 5,643,768. Peptide display libraries, vectors, and screening kits are commercially available from such suppliers as Invitrogen (Carlsbad, CA).

E. Polynucleotides Encoding a Protein Having a Subsequence from a Prototype Polypeptide and is Cross-Reactive to the Prototype Polypeptide

As indicated in (e), above, the present invention provides isolated nucleic acids comprising polynucleotides of the present invention, wherein the polynucleotides encode a protein having a subsequence of contiguous amino acids from a prototype polypeptide of the present invention such as are provided in (a), above. The length of contiguous amino acids from the prototype polypeptide is selected from the group of integers

- 29 -

consisting of from at least 10 to the number of amino acids within the prototype sequence. Thus, for example, the polynucleotide can encode a polypeptide having a subsequence having at least 10, 15, 20, 25, 30, 35, 40, 45, or 50, contiguous amino acids from the prototype polypeptide. Further, the number of such subsequences encoded by a polynucleotide of the instant embodiment can be any integer selected from the group consisting of from 1 to 20, such as 2, 3, 4, or 5. The subsequences can be separated by any integer of nucleotides from 1 to the number of nucleotides in the sequence such as at least 5, 10, 15, 25, 50, 100, or 200 nucleotides.

5

10

15

20

25

30

The proteins encoded by polynucleotides of this embodiment, when presented as an immunogen, elicit the production of polyclonal antibodies which specifically bind to a prototype polypeptide such as but not limited to, a polypeptide encoded by the polynucleotide of (a) or (b), above. Generally, however, a protein encoded by a polynucleotide of this embodiment does not bind to antisera raised against the prototype polypeptide when the antisera has been fully immunosorbed with the prototype polypeptide. Methods of making and assaying for antibody binding specificity/affinity are well known in the art. Exemplary immunoassay formats include ELISA, competitive immunoassays, radioimmunoassays, Western blots, indirect immunofluorescent assays and the like.

In a preferred assay method, fully immunosorbed and pooled antisera which is elicited to the prototype polypeptide can be used in a competitive binding assay to test the protein. The concentration of the prototype polypeptide required to inhibit 50% of the binding of the antisera to the prototype polypeptide is determined. If the amount of the protein required to inhibit binding is less than twice the amount of the prototype protein, then the protein is said to specifically bind to the antisera elicited to the immunogen. Accordingly, the proteins of the present invention embrace allelic variants, conservatively modified variants, and minor recombinant modifications to a prototype polypeptide.

A polynucleotide of the present invention optionally encodes a protein having a molecular weight as the non-glycosylated protein within 20% of the molecular weight of the full-length non-glycosylated polypeptides of the present invention. Molecular weight can be readily determined by SDS-PAGE under reducing conditions. Preferably, the molecular weight is within 15% of a full length polypeptide of the present invention, more preferably within 10% or 5%, and most preferably within 3%, 2%, or 1% of a full

length polypeptide of the present invention. Molecular weight determination of a protein can be conveniently performed by SDS-PAGE under denaturing conditions.

5

10

15

20

Optionally, the polynucleotides of this embodiment will encode a protein having a specific activity at least 50%, 60%, 80%, or 90% of the native, endogenous (i.e., nonisolated), full-length polypeptide of the present invention. Further, the proteins encoded by polynucleotides of this embodiment will optionally have a substantially similar affinity constant (K_m) and/or catalytic activity (i.e., the microscopic rate constant, k_{cat}) as the native endogenous, full-length protein. Those of skill in the art will recognize that $k_{\text{cat}}/K_{\text{m}}$ value determines the specificity for competing substrates and is often referred to as the specificity constant. Proteins of this embodiment can have a k_{cat}/K_m value at least 10% of a non-isolated full-length polypeptide of the present invention as determined using the endogenous substrate of that polypeptide. Optionally, the k_{cat}/K_m value will be at least 20%, 30%, 40%, 50%, and most preferably at least 60%, 70%, 80%, 90%, or 95% the $k_{\text{cat}}/K_{\text{m}}$ value of the non-isolated, full-length polypeptide of the present invention. Determination of $k_{\text{cat}},\,K_{\text{m}}$, and $k_{\text{cat}}/K_{\text{m}}$ can be determined by any number of means well known to those of skill in the art. For example, the initial rates (i.e., the first 5% or less of the reaction) can be determined using rapid mixing and sampling techniques (e.g., continuous-flow, stopped-flow, or rapid quenching techniques), flash photolysis, or relaxation methods (e.g., temperature jumps) in conjunction with such exemplary methods of measuring as spectrophotometry, spectrofluorimetry, nuclear magnetic resonance, or radioactive procedures. Kinetic values are conveniently obtained using a Lineweaver-Burk or Eadie-Hofstee plot.

F. Polynucleotides Complementary to the Polynucleotides of (A)-(E)

As indicated in (f), above, the present invention provides isolated nucleic acids comprising polynucleotides complementary to the polynucleotides of paragraphs A-E, above. As those of skill in the art will recognize, complementary sequences base-pair throughout the entirety of their length with the polynucleotides of (A)-(E) (i.e., have 100% sequence identity over their entire length). Complementary bases associate through hydrogen bonding in double stranded nucleic acids. For example, the following base pairs are complementary: guanine and cytosine; adenine and thymine; and adenine and uracil.

10

15

20

25

G. Polynucleotides Which are Subsequences of the Polynucleotides of (A)-(F)

As indicated in (g), above, the present invention provides isolated nucleic acids comprising polynucleotides which comprise at least 15 contiguous bases from the polynucleotides of (A) through (F) as discussed above. The length of the polynucleotide is given as an integer selected from the group consisting of from at least 15 to the length of the nucleic acid sequence from which the polynucleotide is a subsequence of. Thus, for example, polynucleotides of the present invention are inclusive of polynucleotides comprising at least 15, 20, 25, 30, 40, 50, 60, 75, or 100 contiguous nucleotides in length from the polynucleotides of (A)-(F). Optionally, the number of such subsequences encoded by a polynucleotide of the instant embodiment can be any integer selected from the group consisting of from 1 to 20, such as 2, 3, 4, or 5. The subsequences can be separated by any integer of nucleotides from 1 to the number of nucleotides in the sequence such as at least 5, 10, 15, 25, 50, 100, or 200 nucleotides.

The subsequences of the present invention can comprise structural characteristics of the sequence from which it is derived. Alternatively, the subsequences can lack certain structural characteristics of the larger sequence from which it is derived. For example, a subsequence from a polynucleotide encoding a polypeptide having at least one linear epitope in common with a prototype polypeptide sequence as provided in (a), above, may encode an epitope in common with the prototype sequence. Alternatively, the subsequence may not encode an epitope in common with the prototype sequence but can be used to isolate the larger sequence by, for example, nucleic acid hybridization with the sequence from which it's derived. Subsequences can be used to modulate or detect gene expression by introducing into the subsequences compounds which bind, intercalate, cleave and/or crosslink to nucleic acids. Exemplary compounds include acridine, psoralen, phenanthroline, naphthoquinone, daunomycin or chloroethylaminoaryl conjugates.

Construction of Nucleic Acids

The isolated nucleic acids of the present invention can be made using (a) standard recombinant methods, (b) synthetic techniques, or combinations thereof. In some embodiments, the polynucleotides of the present invention will be cloned, amplified, or otherwise constructed from a monocot. In preferred embodiments the monocot is Zea mays.

PCT/US99/18760

The nucleic acids may conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites may be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences may be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. A polynucleotide of the present invention can be attached to a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention. Additional sequences may be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the polynucleotide into a cell. Typically, the length of a nucleic acid of the present invention less the length of its polynucleotide of the present invention is less than 20 kilobase pairs, often less than 15 kb, and frequently less than 10 kb. Use of cloning vectors, expression vectors, adapters, and linkers is well known and extensivley described in the art. For a description of various nucleic acids see, for example, Stratagene Cloning Systems, Catalogs 1995, 1996, 1997 (La Jolla, CA); and, Amersham Life Sciences, Inc, Catalog '97 (Arlington Heights, IL).

20 A. Recombinant Methods for Constructing Nucleic Acids

The isolated nucleic acid compositions of this invention, such as RNA, cDNA, genomic DNA, or a hybrid thereof, can be obtained from plant biological sources using any number of cloning methodologies known to those of skill in the art. In some embodiments, oligonucleotide probes which selectively hybridize, under stringent conditions, to the polynucleotides of the present invention are used to identify the desired sequence in a cDNA or genomic DNA library. While isolation of RNA, and construction of cDNA and genomic libraries is well known to those of ordinary skill in the art, the following highlights some of the methods employed.

30 A1. mRNA Isolation and Purification

5

10

15

25

Total RNA from plant cells comprises such nucleic acids as mitochondrial RNA, chloroplastic RNA, rRNA, tRNA, hnRNA and mRNA. Total RNA preparation typically involves lysis of cells and removal of proteins, followed by precipitation of nucleic

acids. Extraction of total RNA from plant cells can be accomplished by a variety of means. Frequently, extraction buffers include a strong detergent such as SDS and an organic denaturant such as guanidinium isothiocyanate, guanidine hydrochloride or phenol. Following total RNA isolation, poly(A)⁺ mRNA is typically purified from the remainder RNA using oligo(dT) cellulose. Exemplary total RNA and mRNA isolation protocols are described in *Plant Molecular Biology: A Laboratory Manual*, Clark, Ed., Springer-Verlag, Berlin (1997); and, *Current Protocols in Molecular Biology*, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995). Total RNA and mRNA isolation kits are commercially available from vendors such as Stratagene (La Jolla, CA), Clonetech (Palo Alto, CA), Pharmacia (Piscataway, NJ), and 5'-3' (Paoli, PA). See also, U.S. Patent Nos. 5,614,391; and, 5,459,253. The mRNA can be fractionated into populations with size ranges of about 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0 kb. The cDNA synthesized for each of these fractions can be size selected to the same size range as its mRNA prior to vector insertion. This method helps eliminate truncated cDNA formed by incompletely reverse transcribed mRNA.

A2. Construction of a cDNA Library

5

10

15

20

25

30

Construction of a cDNA library generally entails five steps. First, first strand cDNA synthesis is initiated from a poly(A)⁺ mRNA template using a poly(dT) primer or random hexanucleotides. Second, the resultant RNA-DNA hybrid is converted into double stranded cDNA, typically by a combination of RNAse H and DNA polymerase I (or Klenow fragment). Third, the termini of the double stranded cDNA are ligated to adaptors. Ligation of the adaptors will produce cohesive ends for cloning. Fourth, size selection of the double stranded cDNA eliminates excess adaptors and primer fragments, and eliminates partial cDNA molecules due to degradation of mRNAs or the failure of reverse transcriptase to synthesize complete first strands. Fifth, the cDNAs are ligated into cloning vectors and packaged. cDNA synthesis protocols are well known to the skilled artisan and are described in such standard references as: *Plant Molecular Biology: A Laboratory Manual*, Clark, Ed., Springer-Verlag, Berlin (1997); and, *Current Protocols in Molecular Biology*, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995). cDNA synthesis kits are available from a variety of commercial vendors such as Stratagene or Pharmacia.

PCT/US99/18760

A number of cDNA synthesis protocols have been described which provide substantially pure full-length cDNA libraries. Substantially pure full-length cDNA libraries are constructed to comprise at least 90%, and more preferably at least 93% or 95% full-length inserts amongst clones containing inserts. The length of insert in such libraries can be from 0 to 8, 9, 10, 11, 12, 13, or more kilobase pairs. Vectors to accommodate inserts of these sizes are known in the art and available commercially. See, e.g., Stratagene's lambda ZAP Express (cDNA cloning vector with 0 to 12 kb cloning capacity).

An exemplary method of constructing a greater than 95% pure full-length cDNA library is described by Carninci et al., Genomics, 37:327-336 (1996). In that protocol, the cap-structure of eukaryotic mRNA is chemically labeled with biotin. By using streptavidin-coated magnetic beads, only the full-length first-strand cDNA/mRNA hybrids are selectively recovered after RNase I treatment. The method provides a high yield library with an unbiased representation of the starting mRNA population. Other methods for producing full-length libraries are known in the art. See, e.g., Edery et al., Mol. Cell Biol., 15(6):3363-3371 (1995); and, PCT Application WO 96/34981.

A3. Normalized or Subtracted cDNA Libraries

5

10

15

20

. 25

30

A non-normalized cDNA library represents the mRNA population of the tissue it was made from. Since unique clones are out-numbered by clones derived from highly expressed genes their isolation can be laborious. Normalization of a cDNA library is the process of creating a library in which each clone is more equally represented.

A number of approaches to normalize cDNA libraries are known in the art. One approach is based on hybridization to genomic DNA. The frequency of each hybridized cDNA in the resulting normalized library would be proportional to that of each corresponding gene in the genomic DNA. Another approach is based on kinetics. If cDNA reannealing follows second-order kinetics, rarer species anneal less rapidly and the remaining single-stranded fraction of cDNA becomes progressively more normalized during the course of the hybridization. Specific loss of any species of cDNA, regardless of its abundance, does not occur at any Cot value. Construction of normalized libraries is described in Ko, *Nucl. Acids. Res.*, 18(19):5705-5711 (1990); Patanjali *et al.*, *Proc. Natl. Acad. U.S.A.*, 88:1943-1947 (1991); U.S. Patents 5,482,685, and 5,637,685. In an exemplary method described by Soares *et al.*, normalization resulted in reduction of

PCT/US99/18760

the abundance of clones from a range of four orders of magnitude to a narrow range of only 1 order of magnitude. *Proc. Natl. Acad. Sci. USA*, 91:9228-9232 (1994).

- 35 -

Subtracted cDNA libraries are another means to increase the proportion of less abundant cDNA species. In this procedure, cDNA prepared from one pool of mRNA is depleted of sequences present in a second pool of mRNA by hybridization. The cDNA:mRNA hybrids are removed and the remaining un-hybridized cDNA pool is enriched for sequences unique to that pool. See, Foote et al. in, Plant Molecular Biology: A Laboratory Manual, Clark, Ed., Springer-Verlag, Berlin (1997); Kho and Zarbl, Technique, 3(2):58-63 (1991); Sive and St. John, Nucl. Acids Res., 16(22):10937 (1988); Current Protocols in Molecular Biology, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995); and, Swaroop et al., Nucl. Acids Res., 19)8):1954 (1991). cDNA subtraction kits are commercially available. See, e.g., PCR-Select (Clontech).

15 A4. Construction of a Genomic Library

5

10

20

25

30

To construct genomic libraries, large segments of genomic DNA are generated by random fragmentation, e.g. using restriction endonucleases, and are ligated with vector DNA to form concatemers that can be packaged into the appropriate vector.

Methodologies to accomplish these ends, and sequencing methods to verify the sequence of nucleic acids are well known in the art. Examples of appropriate molecular biological techniques and instructions sufficient to direct persons of skill through many construction, cloning, and screening methodologies are found in Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Vols. 1-3 (1989), Methods in Enzymology, Vol. 152: Guide to Molecular Cloning Techniques, Berger and Kimmel, Eds., San Diego: Academic Press, Inc. (1987), Current Protocols in Molecular Biology, Ausubel, et al., Eds., Greene Publishing and Wiley-Interscience, New York (1995); Plant Molecular Biology: A Laboratory Manual, Clark, Ed., Springer-Verlag, Berlin (1997). Kits for construction of genomic libraries are also commercially available.

A5. Nucleic Acid Screening and Isolation Methods

The cDNA or genomic library can be screened using a probe based upon the sequence of a polynucleotide of the present invention such as those disclosed herein.

- 36 -

Probes may be used to hybridize with genomic DNA or cDNA sequences to isolate homologous genes in the same or different plant species. Those of skill in the art will appreciate that various degrees of stringency of hybridization can be employed in the assay; and either the hybridization or the wash medium can be stringent. As the conditions for hybridization become more stringent, there must be a greater degree of complementarity between the probe and the target for duplex formation to occur. The degree of stringency can be controlled by temperature, ionic strength, pH and the presence of a partially denaturing solvent such as formamide. For example, the stringency of hybridization is conveniently varied by changing the polarity of the reactant solution through manipulation of the concentration of formamide within the range of 0% to 50%. The degree of complementarity (sequence identity) required for detectable binding will vary in accordance with the stringency of the hybridization medium and/or wash medium. The degree of complementarity will optimally be 100 percent; however, it should be understood that minor sequence variations in the probes and primers may be compensated for by reducing the stringency of the hybridization and/or wash medium.

5

10

15

20

25

30

The nucleic acids of interest can also be amplified from nucleic acid samples using amplification techniques. For instance, polymerase chain reaction (PCR) technology can be used to amplify the sequences of polynucleotides of the present invention and related genes directly from genomic DNA or cDNA libraries. PCR and other *in vitro* amplification methods may also be useful, for example, to clone nucleic acid sequences that code for proteins to be expressed, to make nucleic acids to use as probes for detecting the presence of the desired mRNA in samples, for nucleic acid sequencing, or for other purposes. Examples of techniques sufficient to direct persons of skill through *in vitro* amplification methods are found in Berger, Sambrook, and Ausubel, as well as Mullis *et al.*, U.S. Patent No. 4,683,202 (1987); and, *PCR Protocols A Guide to Methods and Applications*, Innis *et al.*, Eds., Academic Press Inc., San Diego, CA (1990). Commercially available kits for genomic PCR amplification are known in the art. See, e.g., Advantage-GC Genomic PCR Kit (Clontech). The T4 gene 32 protein (Boehringer Mannheim) can be used to improve yield of long PCR products.

PCR-based screening methods have also been described. Wilfinger et al. describe a PCR-based method in which the longest cDNA is identified in the first step so that incomplete clones can be eliminated from study. BioTechniques, 22(3): 481-486 (1997). In that method, a primer pair is synthesized with one primer annealing to the 5'

end of the sense strand of the desired cDNA and the other primer to the vector. Clones are pooled to allow large-scale screening. By this procedure, the longest possible clone is identified amongst candidate clones. Further, the PCR product is used solely as a diagnostic for the presence of the desired cDNA and does not utilize the PCR product itself. Such methods are particularly effective in combination with a full-length cDNA construction methodology, above.

B. Synthetic Methods for Constructing Nucleic Acids

5

The isolated nucleic acids of the present invention can also be prepared by direct 10 chemical synthesis by methods such as the phosphotriester method of Narang et al., Meth. Enzymol. 68: 90-99 (1979); the phosphodiester method of Brown et al., Meth. Enzymol. 68: 109-151 (1979); the diethylphosphoramidite method of Beaucage et al., Tetra. Lett. 22: 1859-1862 (1981); the solid phase phosphoramidite triester method described by Beaucage and Caruthers, Tetra. Letts. 22(20): 1859-1862 (1981), e.g., using an automated synthesizer, e.g., as described in Needham-VanDevanter et al., 15 Nucleic Acids Res., 12: 6159-6168 (1984); and, the solid support method of U.S. Patent No. 4,458,066. Chemical synthesis generally produces a single stranded oligonucleotide. This may be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the 20 single strand as a template. One of skill will recognize that while chemical synthesis of DNA is limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

Recombinant Expression Cassettes

The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence coding for the desired polynucleotide of the present invention, for example a cDNA or a genomic sequence encoding a full length polypeptide of the present invention, can be used to construct a recombinant expression cassette which can be introduced into the desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences which will direct the transcription of the polynucleotide in the intended host cell, such as tissues of a transformed plant.

- 38 -

For example, plant expression vectors may include (1) a cloned plant gene under the transcriptional control of 5' and 3' regulatory sequences and (2) a dominant selectable marker. Such plant expression vectors may also contain, if desired, a promoter regulatory region (e.g., one conferring inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific/selective expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

5

10

15

30

A plant promoter fragment can be employed which will direct expression of a polynucleotide of the present invention in all tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the cauliflower mosaic virus (CaMV) 35S transcription initiation region, the 1'- or 2'- promoter derived from T-DNA of Agrobacterium tumefaciens, the ubiquitin 1 promoter, the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Patent No. 5,683,439), the Nos promoter, the pEmu promoter, the rubisco promoter, the GRP1-8 promoter, the actin promoter, the F3.7 promoter, and other transcription initiation regions from various plant genes known to those of skill.

Alternatively, the plant promoter can direct expression of a polynucleotide of the

present invention in a specific tissue or may be otherwise under more precise
environmental or developmental control. Such promoters are referred to here as
"inducible" promoters. Environmental conditions that may effect transcription by
inducible promoters include pathogen attack, anaerobic conditions, or the presence of
light. Examples of inducible promoters are the Adh1 promoter which is inducible by
hypoxia or cold stress, the Hsp70 promoter which is inducible by heat stress, and the
PPDK promoter which is inducible by light.

Examples of promoters under developmental control include promoters that initiate transcription only, or preferentially, in certain tissues, such as leaves, roots, fruit, seeds, or flowers. The operation of a promoter may also vary depending on its location in the genome. Thus, an inducible promoter may become fully or partially constitutive in certain locations.

Both heterologous and non-heterologous (i.e., endogenous) promoters can be employed to direct expression of the nucleic acids of the present invention. These

WO 00/09706

5

10

15

20

25

30

- 39 -

PCT/US99/18760

promoters can also be used, for example, in recombinant expression cassettes to drive expression of antisense nucleic acids to reduce, increase, or alter concentration and/or composition of the proteins of the present invention in a desired tissue. Thus, in some embodiments, the nucleic acid construct will comprise a promoter functional in a plant cell, such as in *Zea mays*, operably linked to a polynucleotide of the present invention. Promoters useful in these embodiments include the endogenous promoters driving expression of a polypeptide of the present invention.

In some embodiments, isolated nucleic acids which serve as promoter or enhancer elements can be introduced in the appropriate position (generally upstream) of a non-heterologous form of a polynucleotide of the present invention so as to up or down regulate expression of a polynucleotide of the present invention. For example, endogenous promoters can be altered *in vivo* by mutation, deletion, and/or substitution (see, Kmiec, U.S. Patent 5,565,350; Zarling *et al.*, PCT/US93/03868), or isolated promoters can be introduced into a plant cell in the proper orientation and distance from a gene of the present invention so as to control the expression of the gene. Gene expression can be modulated under conditions suitable for plant growth so as to alter the total concentration and/or alter the composition of the polypeptides of the present invention in plant cell. Thus, the present invention provides compositions, and methods for making, heterologous promoters and/or enhancers operably linked to a native, endogenous (i.e., non-heterologous) form of a polynucleotide of the present invention.

Methods for identifying promoters with a particular expression pattern, in terms of, e.g., tissue type, cell type, stage of development, and/or environmental conditions, are well known in the art. See, e.g., *The Maize Handbook*, Chapters 114-115, Freeling and Walbot, Eds., Springer, New York (1994); *Corn and Corn Improvement*, 3rd edition, Chapter 6, Sprague and Dudley, Eds., American Society of Agronomy, Madison, Wisconsin (1988). A typical step in promoter isolation methods is identification of gene products that are expressed with some degree of specificity in the target tissue. Amongst the range of methodologies are: differential hybridization to cDNA libraries; subtractive hybridization; differential display; differential 2-D protein gel electrophoresis; DNA probe arrays; and isolation of proteins known to be expressed with some specificity in the target tissue. Such methods are well known to those of skill in the art. Commercially available products for identifying promoters are known in the art such as Clontech's (Palo Alto, CA) Universal GenomeWalker Kit.

- 40 -

For the protein-based methods, it is helpful to obtain the amino acid sequence for at least a portion of the identified protein, and then to use the protein sequence as the basis for preparing a nucleic acid that can be used as a probe to identify either genomic DNA directly, or preferably, to identify a cDNA clone from a library prepared from the target tissue. Once such a cDNA clone has been identified, that sequence can be used to identify the sequence at the 5' end of the transcript of the indicated gene. For differential hybridization, subtractive hybridization and differential display, the nucleic acid sequence identified as enriched in the target tissue is used to identify the sequence at the 5' end of the transcript of the indicated gene. Once such sequences are identified, starting either from protein sequences or nucleic acid sequences, any of these sequences identified as being from the gene transcript can be used to screen a genomic library prepared from the target organism. Methods for identifying and confirming the transcriptional start site are well known in the art.

5

10

15

20

25

30

In the process of isolating promoters expressed under particular environmental conditions or stresses, or in specific tissues, or at particular developmental stages, a number of genes are identified that are expressed under the desired circumstances, in the desired tissue, or at the desired stage. Further analysis will reveal expression of each particular gene in one or more other tissues of the plant. One can identify a promoter with activity in the desired tissue or condition but that do not have activity in any other common tissue.

To identify the promoter sequence, the 5' portions of the clones described here are analyzed for sequences characteristic of promoter sequences. For instance, promoter sequence elements include the TATA box consensus sequence (TATAAT), which is usually an AT-rich stretch of 5-10 bp located approximately 20 to 40 base pairs upstream of the transcription start site. Identification of the TATA box is well known in the art. For example, one way to predict the location of this element is to identify the transcription start site using standard RNA-mapping techniques such as primer extension, S1 analysis, and/or RNase protection. To confirm the presence of the AT-rich sequence, a structure-function analysis can be performed involving mutagenesis of the putative region and quantification of the mutation's effect on expression of a linked downstream reporter gene. See, e.g., *The Maize Handbook*, Chapter 114, Freeling and Walbot, Eds., Springer, New York, (1994).

- 41 -

In plants, further upstream from the TATA box, at positions -80 to -100, there is typically a promoter element (i.e., the CAAT box) with a series of adenines surrounding the trinucleotide G (or T) N G. J. Messing et al., in Genetic Engineering in Plants, Kosage, Meredith and Hollaender, Eds., pp. 221-227 1983. In maize, there is no well conserved CAAT box but there are several short, conserved protein-binding motifs upstream of the TATA box. These include motifs for the trans-acting transcription factors involved in light regulation, anaerobic induction, hormonal regulation, or anthocyanin biosynthesis, as appropriate for each gene.

5

10

15

20

25

30

Once promoter and/or gene sequences are known, a region of suitable size is selected from the genomic DNA that is 5' to the transcriptional start, or the translational start site, and such sequences are then linked to a coding sequence. If the transcriptional start site is used as the point of fusion, any of a number of possible 5' untranslated regions can be used in between the transcriptional start site and the partial coding sequence. If the translational start site at the 3' end of the specific promoter is used, then it is linked directly to the methionine start codon of a coding sequence.

If polypeptide expression is desired, it is generally desirable to include a polyadenylation region at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from the natural gene, from a variety of other plant genes, or from T-DNA. The 3' end sequence to be added can be derived from, for example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene.

An intron sequence can be added to the 5' untranslated region or the coding sequence of the partial coding sequence to increase the amount of the mature message that accumulates in the cytosol. Inclusion of a spliceable intron in the transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold. Buchman and Berg, *Mol. Cell Biol.* 8: 4395-4405 (1988); Callis *et al.*, *Genes Dev.* 1: 1183-1200 (1987). Such intron enhancement of gene expression is typically greatest when placed near the 5' end of the transcription unit. Use of maize introns Adh1-S intron 1, 2, and 6, the Bronze-1 intron are known in the art. See generally, *The Maize Handbook*, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994).

The vector comprising the sequences from a polynucleotide of the present invention will typically comprise a marker gene which confers a selectable phenotype on

- 42 -

plant cells. Usually, the selectable marker gene will encode antibiotic resistance, with suitable genes including genes coding for resistance to the antibiotic spectinomycin (e.g., the aada gene), the streptomycin phosphotransferase (SPT) gene coding for streptomycin resistance, the neomycin phosphotransferase (NPTII) gene encoding kanamycin or geneticin resistance, the hygromycin phosphotransferase (HPT) gene coding for hygromycin resistance, genes coding for resistance to herbicides which act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides which act to inhibit action of glutamine synthase, such as phosphinothricin or basta (e.g., the *bar* gene), or other such genes known in the art. The *bar* gene encodes resistance to the herbicide basta, the *nptII* gene encodes resistance to the antibiotics kanamycin and geneticin, and the ALS gene encodes resistance to the herbicide chlorsulfuron.

5

10

25

30

Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of Agrobacterium tumefaciens described by Rogers et al., Meth. In Enzymol., 153:253-277 (1987). These vectors are plant integrating vectors in that on transformation, the vectors integrate a portion of vector DNA into the genome of the host plant. Exemplary A.

20 tumefaciens vectors useful herein are plasmids pKYLX6 and pKYLX7 of Schardl et al., Gene, 61:1-11 (1987) and Berger et al., Proc. Natl. Acad. Sci. U.S.A., 86:8402-8406 (1989). Another useful vector herein is plasmid pBI101.2 that is available from Clontech Laboratories, Inc. (Palo Alto, CA).

A polynucleotide of the present invention can be expressed in either sense or antisense orientation as desired. It will be appreciated that control of gene expression in either sense or anti-sense orientation can have a direct impact on the observable plant characteristics. Antisense technology can be conveniently used to gene expression in plants. To accomplish this, a nucleic acid segment from the desired gene is cloned and operably linked to a promoter such that the anti-sense strand of RNA will be transcribed. The construct is then transformed into plants and the antisense strand of RNA is produced. In plant cells, it has been shown that antisense RNA inhibits gene expression by preventing the accumulation of mRNA which encodes the enzyme of interest, see,

- 43 -

e.g., Sheehy et al., Proc. Nat'l. Acad. Sci. (USA) 85: 8805-8809 (1988); and Hiatt et al., U.S. Patent No. 4,801,340.

Another method of suppression is sense suppression. Introduction of nucleic acid configured in the sense orientation has been shown to be an effective means by which to block the transcription of target genes. For an example of the use of this method to modulate expression of endogenous genes see, Napoli *et al.*, *The Plant Cell* 2: 279-289 (1990) and U.S. Patent No. 5,034,323.

5

10

15

20

25

30

Catalytic RNA molecules or ribozymes can also be used to inhibit expression of plant genes. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs. The design and use of target RNA-specific ribozymes is described in Haseloff *et al.*, *Nature* 334: 585-591 (1988).

A variety of cross-linking agents, alkylating agents and radical generating species as pendant groups on polynucleotides of the present invention can be used to bind, label, detect, and/or cleave nucleic acids. For example, Vlassov, V. V., et al., Nucleic Acids Res (1986) 14:4065-4076, describe covalent bonding of a single-stranded DNA fragment with alkylating derivatives of nucleotides complementary to target sequences. A report of similar work by the same group is that by Knorre, D. G., et al., Biochimie (1985) 67:785-789. Iverson and Dervan also showed sequence-specific cleavage of singlestranded DNA mediated by incorporation of a modified nucleotide which was capable of activating cleavage (J Am Chem Soc (1987) 109:1241-1243). Meyer, R. B., et al., J Am Chem Soc (1989) 111:8517-8519, effect covalent crosslinking to a target nucleotide using an alkylating agent complementary to the single-stranded target nucleotide sequence. A photoactivated crosslinking to single-stranded oligonucleotides mediated by psoralen was disclosed by Lee, B. L., et al., Biochemistry (1988) 27:3197-3203. Use of crosslinking in triple-helix forming probes was also disclosed by Home, et al., J Am Chem Soc (1990) 112:2435-2437. Use of N4, N4-ethanocytosine as an alkylating agent to crosslink to single-stranded oligonucleotides has also been described by Webb and Matteucci, JAm Chem Soc (1986) 108:2764-2765; Nucleic Acids Res (1986) 14:7661-7674; Feteritz

- 44 .

et al., J. Am. Chem. Soc. 113:4000 (1991). Various compounds to bind, detect, label, and/or cleave nucleic acids are known in the art. See, for example, U.S. Patent Nos. 5,543,507; 5,672,593; 5,484,908; 5,256,648; and, 5,681941.

5 Proteins

10

15

20

25

30

The isolated proteins of the present invention comprise a polypeptide having at least 10 amino acids encoded by any one of the polynucleotides of the present invention as discussed more fully, above, or polypeptides which are conservatively modified variants thereof. The proteins of the present invention or variants thereof can comprise any number of contiguous amino acid residues from a polypeptide of the present invention, wherein that number is selected from the group of integers consisting of from 10 to the number of residues in a full-length polypeptide of the present invention. Optionally, this subsequence of contiguous amino acids is at least 15, 20, 25, 30, 35, or 40 amino acids in length, often at least 50, 60, 70, 80, or 90 amino acids in length. Further, the number of such subsequences can be any integer selected from the group consisting of from 1 to 20, such as 2, 3, 4, or 5.

As those of skill will appreciate, the present invention includes catalytically active polypeptides of the present invention (i.e., enzymes). Catalytically active polypeptides have a specific activity of at least 20%, 30%, or 40%, and preferably at least 50%, 60%, or 70%, and most preferably at least 80%, 90%, or 95% that of the native (nonsynthetic), endogenous polypeptide. Further, the substrate specificity (k_{car}/K_m) is optionally substantially similar to the native (non-synthetic), endogenous polypeptide. Typically, the K_m will be at least 30%, 40%, or 50%, that of the native (non-synthetic), endogenous polypeptide; and more preferably at least 60%, 70%, 80%, or 90%. Methods of assaying and quantifying measures of enzymatic activity and substrate specificity (k_{car}/K_m) , are well known to those of skill in the art.

Generally, the proteins of the present invention will, when presented as an immunogen, elicit production of an antibody specifically reactive to a polypeptide of the present invention. Further, the proteins of the present invention will not bind to antisera raised against a polypeptide of the present invention which has been fully immunosorbed with the same polypeptide. Immunoassays for determining binding are well known to those of skill in the art. A preferred immunoassay is a competitive immunoassay as discussed, *infra*. Thus, the proteins of the present invention can be employed as

WO 00/09706

immunogens for constructing antibodies immunoreactive to a protein of the present invention for such exemplary utilities as immunoassays or protein purification techniques.

5 Expression of Proteins in Host Cells

10

15

20

25

30

Using the nucleic acids of the present invention, one may express a protein of the present invention in a recombinantly engineered cell such as bacteria, yeast, insect, mammalian, or preferably plant cells. The cells produce the protein in a non-natural condition (e.g., in quantity, composition, location, and/or time), because they have been genetically altered through human intervention to do so.

It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made.

In brief summary, the expression of isolated nucleic acids encoding a protein of the present invention will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein of the present invention. To obtain high level expression of a cloned gene, it is desirable to construct expression vectors which contain, at the minimum, a strong promoter to direct transcription, a ribosome binding site for translational initiation, and a transcription/translation terminator. One of skill would recognize that modifications can be made to a protein of the present invention without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids (e.g., poly His) placed on either terminus to create conveniently located purification sequences. Restriction sites or termination codons can also be introduced.

A. Expression in Prokaryotes

Prokaryotic cells may be used as hosts for expression. Prokaryotes most frequently are represented by various strains of *E. coli*; however, other microbial strains may also be used. Commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta lactamase (penicillinase) and lactose (lac) promoter systems (Chang et al., Nature 198:1056 (1977)), the tryptophan (trp) promoter system (Goeddel et al., Nucleic Acids Res. 8:4057 (1980)) and the lambda derived P L promoter and N-gene ribosome binding site (Shimatake *et al.*, Nature 292:128 (1981)). The inclusion of selection markers in DNA vectors transfected in *E. coli* is also useful. Examples of such markers include genes specifying resistance to ampicillin, tetracycline, or chloramphenicol.

The vector is selected to allow introduction into the appropriate host cell. Bacterial vectors are typically of plasmid or phage origin. Appropriate bacterial cells are infected with phage vector particles or transfected with naked phage vector DNA. If a plasmid vector is used, the bacterial cells are transfected with the plasmid vector DNA. Expression systems for expressing a protein of the present invention are available using Bacillus sp. and Salmonella (Palva, et al., Gene 22: 229-235 (1983); Mosbach, et al., Nature 302: 543-545 (1983)).

20

25

30

5

10

15

B. Expression in Eukaryotes

A variety of eukaryotic expression systems such as yeast, insect cell lines, plant and mammalian cells, are known to those of skill in the art. As explained briefly below, a of the present invention can be expressed in these eukaryotic systems. In some embodiments, transformed/transfected plant cells, as discussed *infra*, are employed as expression systems for production of the proteins of the instant invention.

Synthesis of heterologous proteins in yeast is well known. Sherman, F., et al., Methods in Yeast Genetics, Cold Spring Harbor Laboratory (1982) is a well recognized work describing the various methods available to produce the protein in yeast. Two widely utilized yeast for production of eukaryotic proteins are Saccharomyces cerevisiae and Pichia pastoris. Vectors, strains, and protocols for expression in Saccharomyces and Pichia are known in the art and available from commercial suppliers (e.g., Invitrogen). Suitable vectors usually have expression control sequences, such as

- 47 -

promoters, including 3-phosphoglycerate kinase or alcohol oxidase, and an origin of replication, termination sequences and the like as desired.

A protein of the present invention, once expressed, can be isolated from yeast by lysing the cells and applying standard protein isolation techniques to the lysates. The monitoring of the purification process can be accomplished by using Western blot techniques or radioimmunoassay of other standard immunoassay techniques.

5

10

15

20

25

30

The sequences encoding proteins of the present invention can also be ligated to various expression vectors for use in transfecting cell cultures of, for instance, mammalian, insect, or plant origin. Illustrative of cell cultures useful for the production of the peptides are mammalian cells. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions may also be used. A number of suitable host cell lines capable of expressing intact proteins have been developed in the art, and include the HEK293, BHK21, and CHO cell lines. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter (e.g., the CMV promoter, a HSV tk promoter or pgk (phosphoglycerate kinase) promoter), an enhancer (Queen et al., Immunol. Rev. 89: 49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. Other animal cells useful for production of proteins of the present invention are available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (7th edition, 1992).

Appropriate vectors for expressing proteins of the present invention in insect cells are usually derived from the SF9 baculovirus. Suitable insect cell lines include mosquito larvae, silkworm, armyworm, moth and *Drosophila* cell lines such as a Schneider cell line (See Schneider, *J. Embryol. Exp. Morphol.* 27: 353-365 (1987).

As with yeast, when higher animal or plant host cells are employed, polyadenlyation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenlyation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript may also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, et al., J. Virol. 45: 773-781 (1983)). Additionally, gene sequences to control replication in the host cell may be incorporated into the vector such as those found in bovine papilloma virus type-vectors. Saveria-Campo, M., Bovine Papilloma Virus DNA a

- 48 -

Eukaryotic Cloning Vector in *DNA Cloning Vol. II a Practical Approach*, D.M. Glover, Ed., IRL Press, Arlington, Virginia pp. 213-238 (1985).

Transfection/Transformation of Cells

The method of transformation/transfection is not critical to the instant invention; various methods of transformation or transfection are currently available. As newer methods are available to transform crops or other host cells they may be directly applied. Accordingly, a wide variety of methods have been developed to insert a DNA sequence into the genome of a host cell to obtain the transcription and/or translation of the sequence to effect phenotypic changes in the organism. Thus, any method which provides for efficient transformation/transfection may be employed.

A. Plant Transformation

15

A DNA sequence coding for the desired polynucleotide of the present invention, for example a cDNA or a genomic sequence encoding a full length protein, will be used to construct a recombinant expression cassette which can be introduced into the desired plant.

Isolated nucleic acid acids of the present invention can be introduced into plants according techniques known in the art. Generally, recombinant expression cassettes as described above and suitable for transformation of plant cells are prepared. Techniques 20 for transforming a wide variety of higher plant species are well known and described in the technical, scientific, and patent literature. See, for example, Weising et al., Ann. Rev. Genet. 22: 421-477 (1988). For example, the DNA construct may be introduced directly into the genomic DNA of the plant cell using techniques such as electroporation, 25 PEG poration, particle bombardment, silicon fiber delivery, or microinjection of plant cell protoplasts or embryogenic callus. See e.g., Tomes, et al., Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment, pp. 197-213 in Plant Cell, Tissue and Organ Culture, Fundamental Methods, (eds. O.L. Gamborg and G.C. Phillips, Springer-Verlag Berlin Heidelberg New York, 1995). Alternatively, the DNA constructs may be combined with suitable T-DNA flanking regions and introduced into a 30 conventional Agrobacterium tumefaciens host vector. The virulence functions of the Agrobacterium tumefaciens host will direct the insertion of the construct and adjacent

10

marker into the plant cell DNA when the cell is infected by the bacteria. See, U.S. Patent No. 5,591,616.

The introduction of DNA constructs using polyethylene glycol precipitation is described in Paszkowski et al., Embo J. 3: 2717-2722 (1984). Electroporation techniques are described in Fromm et al., Proc. Natl. Acad. Sci. 82: 5824 (1985). Ballistic transformation techniques are described in Klein et al., Nature 327: 70-73 (1987).

Agrobacterium tumefaciens-meditated transformation techniques are well described in the scientific literature. See, for example Horsch et al., Science 233: 496-498 (1984), and Fraley et al., Proc. Natl. Acad. Sci. 80: 4803 (1983). Although Agrobacterium is useful primarily in dicots, certain monocots can be transformed by Agrobacterium. For instance, Agrobacterium transformation of maize is described in U.S. Patent No. 5,550,318.

Other methods of transfection or transformation include (1) Agrobacterium

15 rhizogenes-mediated transformation (see, e.g., Lichtenstein and Fuller In: Genetic Engineering, vol. 6, PWJ Rigby, Ed., London, Academic Press, 1987; and Lichtenstein, C. P., and Draper, J., In: DNA Cloning, Vol. II, D. M. Glover, Ed., Oxford, IRI Press, 1985), Application PCT/US87/02512 (WO 88/02405 published Apr. 7, 1988) describes the use of A. rhizogenes strain A4 and its Ri plasmid along with A. tumefaciens vectors pARC8 or pARC16 (2) liposome-mediated DNA uptake (see, e.g., Freeman et al., Plant Cell Physiol. 25: 1353, 1984), (3) the vortexing method (see, e.g., Kindle, Proc. Natl. Acad. Sci., USA 87: 1228, (1990).

DNA can also be introduced into plants by direct DNA transfer into pollen as described by Zhou et al., Methods in Enzymology, 101:433 (1983); D. Hess, Intern Rev. Cytol., 107:367 (1987); Luo et al., Plane Mol. Biol. Reporter, 6:165 (1988). Expression of polypeptide coding genes can be obtained by injection of the DNA into reproductive organs of a plant as described by Pena et al., Nature, 325.:274 (1987). DNA can also be injected directly into the cells of immature embryos and the rehydration of desiccated embryos as described by Neuhaus et al., Theor. Appl. Genet., 75:30 (1987); and Benbrook et al., in Proceedings Bio Expo 1986, Butterworth, Stoneham, Mass., pp. 27-54 (1986). A variety of plant viruses that can be employed as vectors are known in the art and include cauliflower mosaic virus (CaMV), geminivirus, brome mosaic virus, and tobacco mosaic virus.

- 50 -

B. Transfection of Prokaryotes, Lower Eukaryotes, and Animal Cells

Animal and lower eukaryotic (e.g., yeast) host cells are competent or rendered competent for transfection by various means. There are several well-known methods of introducing DNA into animal cells. These include: calcium phosphate precipitation, fusion of the recipient cells with bacterial protoplasts containing the DNA, treatment of the recipient cells with liposomes containing the DNA, DEAE dextran, electroporation, biolistics, and micro-injection of the DNA directly into the cells. The transfected cells are cultured by means well known in the art. Kuchler, R.J., Biochemical Methods in Cell Culture and Virology, Dowden, Hutchinson and Ross, Inc. (1977).

10

20

30

5

Synthesis of Proteins

The proteins of the present invention can be constructed using non-cellular synthetic methods. Solid phase synthesis of proteins of less than about 50 amino acids in length may be accomplished by attaching the C-terminal amino acid of the sequence to an insoluble support followed by sequential addition of the remaining amino acids in the 15 sequence. Techniques for solid phase synthesis are described by Barany and Merrifield, Solid-Phase Peptide Synthesis, pp. 3-284 in The Peptides: Analysis, Synthesis, Biology. Vol. 2: Special Methods in Peptide Synthesis, Part A.; Merrifield, et al., J. Am. Chem. Soc. 85: 2149-2156 (1963), and Stewart et al., Solid Phase Peptide Synthesis, 2nd ed., Pierce Chem. Co., Rockford, Ill. (1984). Proteins of greater length may be synthesized by condensation of the amino and carboxy termini of shorter fragments. Methods of forming peptide bonds by activation of a carboxy terminal end (e.g., by the use of the coupling reagent N,N'-dicycylohexylcarbodiimide)) is known to those of skill.

25 **Purification of Proteins**

The proteins of the present invention may be purified by standard techniques well known to those of skill in the art. Recombinantly produced proteins of the present invention can be directly expressed or expressed as a fusion protein. The recombinant protein is purified by a combination of cell lysis (e.g., sonication, French press) and affinity chromatography. For fusion products, subsequent digestion of the fusion protein with an appropriate proteolytic enzyme releases the desired recombinant protein.

The proteins of this invention, recombinant or synthetic, may be purified to substantial purity by standard techniques well known in the art, including detergent

- 51 -

solubilization, selective precipitation with such substances as ammonium sulfate, column chromatography, immunopurification methods, and others. See, for instance, R. Scopes, Protein Purification: Principles and Practice, Springer-Verlag: New York (1982); Deutscher, Guide to Protein Purification, Academic Press (1990). For example, antibodies may be raised to the proteins as described herein. Purification from E. coli can be achieved following procedures described in U.S. Patent No. 4,511,503. The protein may then be isolated from cells expressing the protein and further purified by standard protein chemistry techniques as described herein. Detection of the expressed protein is achieved by methods known in the art and include, for example, radioimmunoassays, Western blotting techniques or immunoprecipitation.

Transgenic Plant Regeneration

5

10

15

30

Transformed plant cells which are derived by any of the above transformation techniques can be cultured to regenerate a whole plant which possesses the transformed genotype. Such regeneration techniques often rely on manipulation of certain phytohormones in a tissue culture growth medium, typically relying on a biocide and/or herbicide marker which has been introduced together with a polynucleotide of the present invention. For transformation and regeneration of maize see, Gordon-Kamm et al., The Plant Cell, 2:603-618 (1990).

20 Plants cells transformed with a plant expression vector can be regenerated, e.g., from single cells, callus tissue or leaf discs according to standard plant tissue culture techniques. It is well known in the art that various cells, tissues, and organs from almost any plant can be successfully cultured to regenerate an entire plant. Plant regeneration from cultured protoplasts is described in Evans et al., Protoplasts Isolation and Culture, Handbook of Plant Cell Culture, Macmillilan Publishing Company, New York, pp. 124-25 176 (1983); and Binding, Regeneration of Plants, Plant Protoplasts, CRC Press, Boca Raton, pp. 21-73 (1985).

The regeneration of plants containing the foreign gene introduced by Agrobacterium from leaf explants can be achieved as described by Horsch et al., Science, 227:1229-1231 (1985). In this procedure, transformants are grown in the presence of a selection agent and in a medium that induces the regeneration of shoots in the plant species being transformed as described by Fraley et al., Proc. Natl. Acad. Sci. U.S.A., 80:4803 (1983). This procedure typically produces shoots within two to four

- 52 -

weeks and these transformant shoots are then transferred to an appropriate root-inducing medium containing the selective agent and an antibiotic to prevent bacterial growth.

Transgenic plants of the present invention may be fertile or sterile.

5

10

15

20

25

30

Regeneration can also be obtained from plant callus, explants, organs, or parts thereof. Such regeneration techniques are described generally in Klee et al., Ann. Rev. of Plant Phys. 38: 467-486 (1987). The regeneration of plants from either single plant protoplasts or various explants is well known in the art. See, for example, Methods for Plant Molecular Biology, A. Weissbach and H. Weissbach, eds., Academic Press, Inc., San Diego, Calif. (1988). This regeneration and growth process includes the steps of selection of transformant cells and shoots, rooting the transformant shoots and growth of the plantlets in soil. For maize cell culture and regeneration see generally, The Maize Handbook, Freeling and Walbot, Eds., Springer, New York (1994); Corn and Corn Improvement, 3rd edition, Sprague and Dudley Eds., American Society of Agronomy, Madison, Wisconsin (1988).

One of skill will recognize that after the recombinant expression cassette is stably incorporated in transgenic plants and confirmed to be operable, it can be introduced into other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

In vegetatively propagated crops, mature transgenic plants can be propagated by the taking of cuttings or by tissue culture techniques to produce multiple identical plants. Selection of desirable transgenics is made and new varieties are obtained and propagated vegetatively for commercial use. In seed propagated crops, mature transgenic plants can be self crossed to produce a homozygous inbred plant. The inbred plant produces seed containing the newly introduced heterologous nucleic acid. These seeds can be grown to produce plants that would produce the selected phenotype.

Parts obtained from the regenerated plant, such as flowers, seeds, leaves, branches, fruit, and the like are included in the invention, provided that these parts comprise cells comprising the isolated nucleic acid of the present invention. Progeny and variants, and mutants of the regenerated plants are also included within the scope of the invention, provided that these parts comprise the introduced nucleic acid sequences.

Transgenic plants expressing the selectable marker can be screened for transmission of the nucleic acid of the present invention by, for example, standard immunoblot and DNA detection techniques. Transgenic lines are also typically evaluated

- 53 -

on levels of expression of the heterologous nucleic acid. Expression at the RNA level can be determined initially to identify and quantitate expression-positive plants. Standard techniques for RNA analysis can be employed and include PCR amplification assays using oligonucleotide primers designed to amplify only the heterologous RNA templates and solution hybridization assays using heterologous nucleic acid-specific probes. The RNA-positive plants can then analyzed for protein expression by Western immunoblot analysis using the specifically reactive antibodies of the present invention. In addition, in situ hybridization and immunocytochemistry according to standard protocols can be done using heterologous nucleic acid specific polynucleotide probes and antibodies, respectively, to localize sites of expression within transgenic tissue. Generally, a number of transgenic lines are usually screened for the incorporated nucleic acid to identify and select plants with the most appropriate expression profiles.

A preferred embodiment is a transgenic plant that is homozygous for the added heterologous nucleic acid; i.e., a transgenic plant that contains two added nucleic acid sequences, one gene at the same locus on each chromosome of a chromosome pair. A homozygous transgenic plant can be obtained by sexually mating (selfing) a heterozygous transgenic plant that contains a single added heterologous nucleic acid, germinating some of the seed produced and analyzing the resulting plants produced for altered expression of a polynucleotide of the present invention relative to a control plant (i.e., native, non-transgenic). Back-crossing to a parental plant and out-crossing with a non-transgenic plant are also contemplated.

Modulating Polypeptide Levels and/or Composition

5

10

15

20

25

30

The present invention further provides a method for modulating (i.e., increasing or decreasing) the concentration or composition of the polypeptides of the present invention in a plant or part thereof. Modulation can be effected by increasing or decreasing the concentration and/or the composition (i.e., the ratio of the polypeptides of the present invention) in a plant. The method comprises transforming a plant cell with a recombinant expression cassette comprising a polynucleotide of the present invention as described above to obtain a transformed plant cell, growing the transformed plant cell under plant forming conditions, and inducing expression of a polynucleotide of the present invention in the plant for a time sufficient to modulate concentration and/or composition in the plant or plant part.

- 54 -

In some embodiments, the content and/or composition of polypeptides of the present invention in a plant may be modulated by altering, in vivo or in vitro, the promoter of a non-isolated gene of the present invention to up- or down-regulate gene expression. In some embodiments, the coding regions of native genes of the present invention can be altered via substitution, addition, insertion, or deletion to decrease activity of the encoded enzyme. See, e.g., Kmiec, U.S. Patent 5,565,350; Zarling et al., PCT/US93/03868. And in some embodiments, an isolated nucleic acid (e.g., a vector) comprising a promoter sequence is transfected into a plant cell. Subsequently, a plant cell comprising the promoter operably linked to a polynucleotide of the present invention is selected for by means known to those of skill in the art such as, but not limited to, Southern blot, DNA sequencing, or PCR analysis using primers specific to the promoter and to the gene and detecting amplicons produced therefrom. A plant or plant part altered or modified by the foregoing embodiments is grown under plant forming conditions for a time sufficient to modulate the concentration and/or composition of polypeptides of the present invention in the plant. Plant forming conditions are well known in the art and discussed briefly, above.

In general, concentration or composition is increased or decreased by at least 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% relative to a native control plant, plant part, or cell lacking the aforementioned recombinant expression cassette. Modulation in the present invention may occur during and/or subsequent to growth of the plant to the desired stage of development. Modulating nucleic acid expression temporally and/or in particular tissues can be controlled by employing the appropriate promoter operably linked to a polynucleotide of the present invention in, for example, sense or antisense orientation as discussed in greater detail, *above*. Induction of expression of a polynucleotide of the present invention can also be controlled by exogenous administration of an effective amount of inducing compound. Inducible promoters and inducing compounds which activate expression from these promoters are well known in the art. In preferred embodiments, the polypeptides of the present invention are modulated in monocots, particularly maize.

25

5

10

15

20

- 55 -

The present invention provides a method of genotyping a plant comprising a polynucleotide of the present invention. Preferably, the plant is a monocot, such as maize or sorghum. Genotyping provides a means of distinguishing homologs of a chromosome pair and can be used to differentiate segregants in a plant population.

5

10

15

20

25

30

Molecular marker methods can be used for phylogenetic studies, characterizing genetic relationships among crop varieties, identifying crosses or somatic hybrids, localizing chromosomal segments affecting monogenic traits, map based cloning, and the study of quantitative inheritance. See, e.g., *Plant Molecular Biology: A Laboratory Manual*, Chapter 7, Clark, Ed., Springer-Verlag, Berlin (1997). For molecular marker methods, see generally, The DNA Revolution by Andrew H. Paterson 1996 (Chapter 2) in: Genome Mapping in Plants (ed. Andrew H. Paterson) by Academic Press/R. G. Landis Company, Austin, Texas, pp.7-21.

The particular method of genotyping in the present invention may employ any number of molecular marker analytic techniques such as, but not limited to, restriction fragment length polymorphisms (RFLPs). RFLPs are the product of allelic differences between DNA restriction fragments caused by nucleotide sequence variability. As is well known to those of skill in the art, RFLPs are typically detected by extraction of genomic DNA and digestion with a restriction enzyme. Generally, the resulting fragments are separated according to size and hybridized with a probe; single copy probes are preferred. Restriction fragments from homologous chromosomes are revealed. Differences in fragment size among alleles represent an RFLP. Thus, the present invention further provides a means to follow segregation of a gene or nucleic acid of the present invention as well as chromosomal sequences genetically linked to these genes or nucleic acids using such techniques as RFLP analysis. Linked chromosomal sequences are within 50 centiMorgans (cM), often within 40 or 30 cM, preferably within 20 or 10 cM, more preferably within 5, 3, 2, or 1 cM of a gene of the present invention.

In the present invention, the nucleic acid probes employed for molecular marker mapping of plant nuclear genomes selectively hybridize, under selective hybridization conditions, to a gene encoding a polynucleotide of the present invention. In preferred embodiments, the probes are selected from polynucleotides of the present invention. Typically, these probes are cDNA probes or *Pst I* genomic clones. The length of the probes is discussed in greater detail, above, but are typically at least 15 bases in length,

- 56 -

more preferably at least 20, 25, 30, 35, 40, or 50 bases in length. Generally, however, the probes are less than about 1 kilobase in length. Preferably, the probes are single copy probes that hybridize to a unique locus in a haploid chromosome complement. Some exemplary restriction enzymes employed in RFLP mapping are *EcoRI*, *EcoRv*, and *SstI*. As used herein the term "restriction enzyme" includes reference to a composition that recognizes and, alone or in conjunction with another composition, cleaves at a specific nucleotide sequence.

The method of detecting an RFLP comprises the steps of (a) digesting genomic DNA of a plant with a restriction enzyme; (b) hybridizing a nucleic acid probe, under selective hybridization conditions, to a sequence of a polynucleotide of the present of said genomic DNA; (c) detecting therefrom a RFLP. Other methods of differentiating polymorphic (allelic) variants of polynucleotides of the present invention can be had by utilizing molecular marker techniques well known to those of skill in the art including such techniques as: 1) single stranded conformation analysis (SSCP); 2) denaturing gradient gel electrophoresis (DGGE); 3) RNase protection assays; 4) allele-specific oligonucleotides (ASOs); 5) the use of proteins which recognize nucleotide mismatches, such as the E. coli mutS protein; and 6) allele-specific PCR. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE); heteroduplex analysis (HA); and chemical mismatch cleavage (CMC). Exemplary polymorphic variants are provided in Table I, above. Thus, the present invention further provides a method of genotyping comprising the steps of contacting, under stringent hybridization conditions, a sample suspected of comprising a polynucleotide of the present invention with a nucleic acid probe. Generally, the sample is a plant sample; preferably, a sample suspected of comprising a maize polynucleotide of the present invention (e.g., gene, mRNA). The nucleic acid probe selectively hybridizes, under stringent conditions, to a subsequence of a polynucleotide of the present invention comprising a polymorphic marker. Selective hybridization of the nucleic acid probe to the polymorphic marker nucleic acid sequence yields a hybridization complex. Detection of the hybridization complex indicates the presence of that polymorphic marker in the sample. In preferred embodiments, the nucleic acid probe comprises a polynucleotide of the present invention.

UTR's and Codon Preference

5

10

15

20

25

30

- 57 -

In general, translational efficiency has been found to be regulated by specific sequence elements in the 5' non-coding or untranslated region (5' UTR) of the RNA. Positive sequence motifs include translational initiation consensus sequences (Kozak, Nucleic Acids Res. 15:8125 (1987)) and the 7-methylguanosine cap structure (Drummond et al., Nucleic Acids Res. 13:7375 (1985)). Negative elements include stable intramolecular 5' UTR stem-loop structures (Muesing et al., Cell 48:691 (1987)) and AUG sequences or short open reading frames preceded by an appropriate AUG in the 5' UTR (Kozak, above, Rao et al., Mol. and Cell. Biol. 8:284 (1988)). Accordingly, the present invention provides 5' and/or 3' UTR regions for modulation of translation of heterologous coding sequences.

Further, the polypeptide-encoding segments of the polynucleotides of the present invention can be modified to alter codon usage. Altered codon usage can be employed to alter translational efficiency and/or to optimize the coding sequence for expression in a desired host or to optimize the codon usage in a heterologous sequence for expression in maize. Codon usage in the coding regions of the polynucleotides of the present invention can be analyzed statistically using commercially available software packages such as "Codon Preference" available from the University of Wisconsin Genetics Computer Group (see Devereaux et al., Nucleic Acids Res. 12: 387-395 (1984)) or MacVector 4.1 (Eastman Kodak Co., New Haven, Conn.). Thus, the present invention provides a codon usage frequency characteristic of the coding region of at least one of the polynucleotides of the present invention. The number of polynucleotides that can be used to determine a codon usage frequency can be any integer from 1 to the number of polynucleotides of the present invention as provided herein. Optionally, the polynucleotides will be full-length sequences. An exemplary number of sequences for statistical analysis can be at least 1, 5, 10, 20, 50, or 100.

Sequence Shuffling

5

10

15

20

25

The present invention provides methods for sequence shuffling using polynucleotides of the present invention, and compositions resulting therefrom.

Sequence shuffling is described in PCT publication No. 96/19256. See also, Zhang, J.-H., et al. Proc. Natl. Acad. Sci. USA 94:4504-4509 (1997). Generally, sequence shuffling provides a means for generating libraries of polynucleotides having a desired characteristic which can be selected or screened for. Libraries of recombinant

- 58 -

polynucleotides are generated from a population of related sequence polynucleotides which comprise sequence regions which have substantial sequence identity and can be homologously recombined in vitro or in vivo. The population of sequence-recombined polynucleotides comprises a subpopulation of polynucleotides which possess desired or advantageous characteristics and which can be selected by a suitable selection or screening method. The characteristics can be any property or attribute capable of being selected for or detected in a screening system, and may include properties of: an encoded protein, a transcriptional element, a sequence controlling transcription, RNA processing, RNA stability, chromatin conformation, translation, or other expression property of a gene or transgene, a replicative element, a protein-binding element, or the like, such as any feature which confers a selectable or detectable property. In some embodiments, the selected characteristic will be a decreased K_{m} and/or increased K_{cat} over the wild-type protein as provided herein. In other embodiments, a protein or polynculeotide generated from sequence shuffling will have a ligand binding affinity greater than the non-shuffled wild-type polynucleotide. The increase in such properties can be at least 110%, 120%, 130%, 140% or at least 150% of the wild-type value.

Generic and Consensus Sequences

5

10

15

20

25

30

Polynuclotides and polypeptides of the present invention further include those having: (a) a generic sequence of at least two homologous polynucleotides or polypeptides, respectively, of the present invention; and, (b) a consensus sequence of at least three homologous polynucleotides or polypeptides, respectively, of the present invention. The generic sequence of the present invention comprises each species of polypeptide or polynucleotide embraced by the generic polypeptide or polynucleotide, sequence, respectively. The individual species encompassed by a polynucleotide having an amino acid or nucleic acid consensus sequence can be used to generate antibodies or produce nucleic acid probes or primers to screen for homologs in other species, genera, families, orders, classes, phyla, or kingdoms. For example, a polynucleotide having a consensus sequences from a gene family of *Zea mays* can be used to generate antibody or nucleic acid probes or primers to other *Gramineae* species such as wheat, rice, or sorghum. Alternatively, a polynucleotide having a consensus sequence generated from orthologous genes can be used to identify or isolate orthologs of other taxa. Typically, a polynucleotide having a consensus sequence will be at least 9, 10, 15, 20, 25, 30, or 40

- 59 -

amino acids in length, or 20, 30, 40, 50, 100, or 150 nucleotides in length. As those of skill in the art are aware, a conservative amino acid substitution can be used for amino acids which differ amongst aligned sequence but are from the same conservative substitution group as discussed above. Optionally, no more than 1 or 2 conservative amino acids are substituted for each 10 amino acid length of consensus sequence.

Similar sequences used for generation of a consensus or generic sequence include any number and combination of allelic variants of the same gene, orthologous, or paralogous sequences as provided herein. Optionally, similar sequences used in generating a consensus or generic sequence are identified using the BLAST algorithm's smallest sum probability (P(N)). Various suppliers of sequence-analysis software are listed in chapter 7 of Current Protocols in Molecular Biology, F.M. Ausubel et al., Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (Supplement 30). A polynucleotide sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, or 0.001, and most preferably less than about 0.0001, or 0.00001. Similar polynucleotides can be aligned and a consensus or generic sequence generated using multiple sequence alignment software available from a number of commercial suppliers such as the Genetics Computer Group's (Madison, WI) PILEUP software, Vector NTI's (North Bethesda, MD) ALIGNX, or Genecode's (Ann Arbor, MI) SEQUENCHER. Conveniently, default parameters of such software can be used to generate consensus or generic sequences.

Detection of Nucleic Acids

5

10

15

20

25

30

The present invention further provides methods for detecting a polynucleotide of the present invention in a nucleic acid sample suspected of comprising a polynucleotide of the present invention, such as a plant cell lysate, particularly a lysate of corn. In some embodiments, a gene of the present invention or portion thereof can be amplified prior to the step of contacting the nucleic acid sample with a polynucleotide of the present invention. The nucleic acid sample is contacted with the polynucleotide to form a hybridization complex. The polynucleotide hybridizes under stringent conditions to a gene encoding a polypeptide of the present invention. Formation of the hybridization complex is used to detect a gene encoding a polypeptide of the present invention in the

nucleic acid sample. Those of skill will appreciate that an isolated nucleic acid comprising a polynucleotide of the present invention should lack cross-hybridizing sequences in common with non-target genes that would yield a false positive result.

Detection of the hybridization complex can be achieved using any number of well known methods. For example, the nucleic acid sample, or a portion thereof, may be 5 assayed by hybridization formats including but not limited to, solution phase, solid phase, mixed phase, or in situ hybridization assays. Briefly, in solution (or liquid) phase hybridizations, both the target nucleic acid and the probe or primer are free to interact in the reaction mixture. In solid phase hybridization assays, probes or primers are typically linked to a solid support where they are available for hybridization with target nucleic in solution. In mixed phase, nucleic acid intermediates in solution hybridize to target nucleic acids in solution as well as to a nucleic acid linked to a solid support. In in situ hybridization, the target nucleic acid is liberated from its cellular surroundings in such as to be available for hybridization within the cell while preserving the cellular morphology for subsequent interpretation and analysis. The following articles provide an overview of the various hybridization assay formats: Singer et al., Biotechniques 4(3): 230-250 (1986); Haase et al., Methods in Virology, Vol. VII, pp. 189-226 (1984); Wilkinson, The theory and practice of in situ hybridization in: In situ Hybridization, D.G. Wilkinson, Ed., IRL Press, Oxford University Press, Oxford; and Nucleic Acid Hybridization: A Practical Approach, Hames, B.D. and Higgins, S.J., Eds., IRL Press (1987).

Nucleic Acid Labels and Detection Methods

10

15

20

25

30

The means by which nucleic acids of the present invention are labeled is not a critical aspect of the present invention and can be accomplished by any number of methods currently known or later developed. Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, radioisotopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include biotin for staining with labeled streptavidin conjugate, magnetic beads, fluorescent dyes (e.g., fluorescein, texas red, rhodamine, green fluorescent protein, and the like), radiolabels (e.g., ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P), enzymes (e.g., horse radish peroxidase, alkaline phosphatase and others commonly used

5

10

15

- 61 -

in an ELISA), and colorimetric labels such as colloidal gold or colored glass or plastic (e.g., polystyrene, polypropylene, latex, etc.) beads.

Nucleic acids of the present invention can be labeled by any one of several methods typically used to detect the presence of hybridized nucleic acids. One common method of detection is the use of autoradiography using probes labeled with ³H, ¹²⁵I, ³⁵S, ¹⁴C, or ³²P, or the like. The choice of radio-active isotope depends on research preferences due to ease of synthesis, stability, and half lives of the selected isotopes. Other labels include ligands which bind to antibodies labeled with fluorophores, chemiluminescent agents, and enzymes. Alternatively, probes can be conjugated directly with labels such as fluorophores, chemiluminescent agents or enzymes. The choice of label depends on sensitivity required, ease of conjugation with the probe, stability requirements, and available instrumentation. Labeling the nucleic acids of the present invention is readily achieved such as by the use of labeled PCR primers.

In some embodiments, the label is simultaneously incorporated during the amplification step in the preparation of the nucleic acids. Thus, for example, polymerase chain reaction (PCR) with labeled primers or labeled nucleotides will provide a labeled amplification product. In another embodiment, transcription amplification using a labeled nucleotide (e.g., fluorescein-labeled UTP and/or CTP) incorporates a label into the transcribed nucleic acids.

20 Non-radioactive probes are often labeled by indirect means. For example, a ligand molecule is covalently bound to the probe. The ligand then binds to an anti-ligand molecule which is either inherently detectable or covalently bound to a detectable signal system, such as an enzyme, a fluorophore, or a chemiluminescent compound. Enzymes of interest as labels will primarily be hydrolases, such as phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds 25 include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescers include luciferin, and 2,3dihydrophthalazinediones, e.g., luminol. Ligands and anti-ligands may be varied widely. Where a ligand has a natural anti-ligand, namely ligands such as biotin, thyroxine, and cortisol, it can be used in conjunction with its labeled, naturally occurring 30 anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody.

Probes can also be labeled by direct conjugation with a label. For example, cloned DNA probes have been coupled directly to horseradish peroxidase or alkaline phosphatase, (Renz. M., and Kurz, K., A Colorimetric Method for DNA Hybridization, Nucl. Acids Res. 12: 3435-3444 (1984)) and synthetic oligonucleotides have been coupled directly with alkaline phosphatase (Jablonski, E., et al., Preparation of Oligodeoxynucleotide-Alkaline Phosphatase Conjugates and Their Use as Hybridization Probes, Nuc. Acids. Res. 14: 6115-6128 (1986); and Li P., et al., Enzyme-linked Synthetic Oligonucleotide probes: Non-Radioactive Detection of Enterotoxigenic Escherichia Coli in Faeca Specimens, Nucl. Acids Res. 15: 5275-5287 (1987)).

Means of detecting such labels are well known to those of skill in the art. Thus, for example, radiolabels may be detected using photographic film or scintillation counters, fluorescent markers may be detected using a photodetector to detect emitted light. Enzymatic labels are typically detected by providing the enzyme with a substrate and detecting the reaction product produced by the action of the enzyme on the substrate, and colorimetric labels are detected by simply visualizing the colored label.

Antibodies to Proteins

5

10

15

20

25

30

Antibodies can be raised to a protein of the present invention, including individual, allelic, strain, or species variants, and fragments thereof, both in their naturally occurring (full-length) forms and in recombinant forms. Additionally, antibodies are raised to these proteins in either their native configurations or in non-native configurations. Anti-idiotypic antibodies can also be generated. Many methods of making antibodies are known to persons of skill. The following discussion is presented as a general overview of the techniques available; however, one of skill will recognize that many variations upon the following methods are known.

A number of immunogens are used to produce antibodies specifically reactive with a protein of the present invention. An isolated recombinant, synthetic, or native polynucleotide of the present invention are the preferred immunogens (antigen) for the production of monoclonal or polyclonal antibodies. Those of skill will readily understand that the proteins of the present invention are typically denatured, and optionally reduced, prior to formation of antibodies for screening expression libraries or other assays in which a putative protein of the present invention is expressed or

- 63 -

denatured in a non-native secondary, tertiary, or quartenary structure. Non-isolated polypeptides of the present invention can be used either in pure or impure form.

5

10

15

20

25

30

The protein of the present invention is then injected into an animal capable of producing antibodies. Either monoclonal or polyclonal antibodies can be generated for subsequent use in immunoassays to measure the presence and quantity of the protein of the present invention. Methods of producing polyclonal antibodies are known to those of skill in the art. In brief, an immunogen (antigen), preferably a purified protein, a protein coupled to an appropriate carrier (e.g., GST, keyhole limpet hemanocyanin, etc.), or a protein incorporated into an immunization vector such as a recombinant vaccinia virus (see, U.S. Patent No. 4,722,848) is mixed with an adjuvant and animals are immunized with the mixture. The animal's immune response to the immunogen preparation is monitored by taking test bleeds and determining the titer of reactivity to the protein of interest. When appropriately high titers of antibody to the immunogen are obtained, blood is collected from the animal and antisera are prepared. Further fractionation of the antisera to enrich for antibodies reactive to the protein is performed where desired (See, e.g., Coligan, Current Protocols in Immunology, Wiley/Greene, NY (1991); and Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Press, NY (1989)).

Antibodies, including binding fragments and single chain recombinant versions thereof, against predetermined fragments of a protein of the present invention are raised by immunizing animals, e.g., with conjugates of the fragments with carrier proteins as described above. Typically, the immunogen of interest is a protein of at least about 5 amino acids, more typically the protein is 10 amino acids in length, preferably, 15 amino acids in length and more preferably the protein is 20 amino acids in length or greater. The peptides are typically coupled to a carrier protein (e.g., as a fusion protein), or are recombinantly expressed in an immunization vector. Antigenic determinants on peptides to which antibodies bind are typically 3 to 10 amino acids in length.

Monoclonal antibodies are prepared from cells secreting the desired antibody. Monoclonals antibodies are screened for binding to a protein from which the immunogen was derived. Specific monoclonal and polyclonal antibodies will usually have an antibody binding site with an affinity constant for its cognate monovalent antigen at least between 10⁶-10⁷, usually at least 10⁸, preferably at least 10⁹, more preferably at least 10¹⁰, and most preferably at least 10¹¹ liters/mole.

5

10

15

In some instances, it is desirable to prepare monoclonal antibodies from various mammalian hosts, such as mice, rodents, primates, humans, etc. Description of techniques for preparing such monoclonal antibodies are found in, e.g., Basic and Clinical Immunology, 4th ed., Stites et al., Eds., Lange Medical Publications, Los Altos, CA, and references cited therein; Harlow and Lane, Supra; Goding, Monoclonal Antibodies: Principles and Practice, 2nd ed., Academic Press, New York, NY (1986); and Kohler and Milstein, Nature 256: 495-497 (1975). Summarized briefly, this method proceeds by injecting an animal with an immunogen comprising a protein of the present invention. The animal is then sacrificed and cells taken from its spleen, which are fused with myeloma cells. The result is a hybrid cell or "hybridoma" that is capable of reproducing in vitro. The population of hybridomas is then screened to isolate individual clones, each of which secrete a single antibody species to the immunogen. In this manner, the individual antibody species obtained are the products of immortalized and cloned single B cells from the immune animal generated in response to a specific site recognized on the immunogenic substance.

Other suitable techniques involve selection of libraries of recombinant antibodies in phage or similar vectors (see, e.g., Huse et al., Science 246: 1275-1281 (1989); and Ward, et al., Nature 341: 544-546 (1989); and Vaughan et al., Nature Biotechnology, 14: 309-314 (1996)). Alternatively, high avidity human monoclonal antibodies can be obtained from transgenic mice comprising fragments of the unrearranged human heavy and light chain Ig loci (i.e., minilocus transgenic mice). Fishwild et al., Nature Biotech., 14: 845-851 (1996). Also, recombinant immunoglobulins may be produced. See, Cabilly, U.S. Patent No. 4,816,567; and Queen et al., Proc. Nat'l Acad. Sci. 86: 10029-10033 (1989).

The antibodies of this invention are also used for affinity chromatography in isolating proteins of the present invention. Columns are prepared, e.g., with the antibodies linked to a solid support, e.g., particles, such as agarose, Sephadex, or the like, where a cell lysate is passed through the column, washed, and treated with increasing concentrations of a mild denaturant, whereby purified protein are released.

The antibodies can be used to screen expression libraries for particular expression products such as normal or abnormal protein. Usually the antibodies in such a procedure are labeled with a moiety allowing easy detection of presence of antigen by antibody binding.

WO 00/09706

Antibodies raised against a protein of the present invention can also be used to raise anti-idiotypic antibodies. These are useful for detecting or diagnosing various pathological conditions related to the presence of the respective antigens.

Frequently, the proteins and antibodies of the present invention will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionucleotides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like.

10

5

Protein Immunoassays

Means of detecting the proteins of the present invention are not critical aspects of the present invention. In a preferred embodiment, the proteins are detected and/or quantified using any of a number of well recognized immunological binding assays (see, e.g., U.S. Patents 4,366,241; 4,376,110; 4,517,288; and 4,837,168). For a review of 15 the general immunoassays, see also Methods in Cell Biology, Vol. 37: Antibodies in Cell Biology, Asai, Ed., Academic Press, Inc. New York (1993); Basic and Clinical Immunology 7th Edition, Stites & Terr, Eds. (1991). Moreover, the immunoassays of the present invention can be performed in any of several configurations, e.g., those reviewed in Enzyme Immunoassay, Maggio, Ed., CRC Press, Boca Raton, Florida 20 (1980); Tijan, Practice and Theory of Enzyme Immunoassays, Laboratory Techniques in Biochemistry and Molecular Biology, Elsevier Science Publishers B.V., Amsterdam (1985); Harlow and Lane, above; Immunoassay: A Practical Guide, Chan, Ed., Academic Press, Orlando, FL (1987); Principles and Practice of Immunoassaysm, Price and Newman Eds., Stockton Press, NY (1991); and Non-isotopic Immunoassays, Ngo, 25 Ed., Plenum Press, NY (1988). Immunological binding assays (or immunoassays) typically utilize a "capture agent" to specifically bind to and often immobilize the analyte (in this case, a protein of the present invention). The capture agent is a moiety that specifically binds to the analyte. In a preferred embodiment, the capture agent is an antibody that specifically binds a protein(s) of the present invention. The antibody may 30 be produced by any of a number of means known to those of skill in the art as described herein.

- 66 -

Immunoassays also often utilize a labeling agent to specifically bind to and label the binding complex formed by the capture agent and the analyte. The labeling agent may itself be one of the moieties comprising the antibody/analyte complex. Thus, the labeling agent may be a labeled protein of the present invention or a labeled antibody specifically reactive to a protein of the present invention. Alternatively, the labeling agent may be a third moiety, such as another antibody, that specifically binds to the antibody/protein complex.

5

10

15

20

25

30

In a preferred embodiment, the labeling agent is a second antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

Other proteins capable of specifically binding immunoglobulin constant regions, such as protein A or protein G may also be used as the label agent. These proteins are normal constituents of the cell walls of streptococcal bacteria. They exhibit a strong non-immunogenic reactivity with immunoglobulin constant regions from a variety of species (See, generally Kronval, et al., J. Immunol. 111: 1401-1406 (1973), and Akerstrom, et al., J. Immunol. 135: 2589-2542 (1985)).

Throughout the assays, incubation and/or washing steps may be required after each combination of reagents. Incubation steps can vary from about 5 seconds to several hours, preferably from about 5 minutes to about 24 hours. However, the incubation time will depend upon the assay format, analyte, volume of solution, concentrations, and the like. Usually, the assays will be carried out at ambient temperature, although they can be conducted over a range of temperatures, such as 10°C to 40°C.

While the details of the immunoassays of the present invention may vary with the particular format employed, the method of detecting a protein of the present invention in a biological sample generally comprises the steps of contacting the biological sample with an antibody which specifically reacts, under immunologically reactive conditions, to a protein of the present invention. The antibody is allowed to bind to the protein under immunologically reactive conditions, and the presence of the bound antibody is detected directly or indirectly.

- 67 -

A. Non-Competitive Assay Formats

Immunoassays for detecting proteins of the present invention include competitive and noncompetitive formats. Noncompetitive immunoassays are assays in which the amount of captured analyte (i.e., a protein of the present invention) is directly measured. In one preferred "sandwich" assay, for example, the capture agent (e.g., an antibody specifically reactive, under immunoreactive conditions, to a protein of the present invention) can be bound directly to a solid substrate where they are immobilized. These immobilized antibodies then capture the protein present in the test sample. The protein thus immobilized is then bound by a labeling agent, such as a second antibody bearing a label. Alternatively, the second antibody may lack a label, but it may, in turn, be bound by a labeled third antibody specific to antibodies of the species from which the second antibody is derived. The second can be modified with a detectable moiety, such as biotin, to which a third labeled molecule can specifically bind, such as enzyme-labeled streptavidin.

15

20

30

10

5

B. Competitive Assay Formats

In competitive assays, the amount of analyte present in the sample is measured indirectly by measuring the amount of an added (exogenous) analyte (e.g., a protein of the present invention) displaced (or competed away) from a capture agent (e.g., an antibody specifically reactive, under immunoreactive conditions, to the protein) by the analyte present in the sample. In one competitive assay, a known amount of analyte is added to the sample and the sample is then contacted with a capture agent that specifically binds a protein of the present invention. The amount of protein bound to the capture agent is inversely proportional to the concentration of analyte present in the sample.

25

In a particularly preferred embodiment, the antibody is immobilized on a solid substrate. The amount of protein bound to the antibody may be determined either by measuring the amount of protein present in a protein/antibody complex, or alternatively by measuring the amount of remaining uncomplexed protein. The amount of protein may be detected by providing a labeled protein.

A hapten inhibition assay is another preferred competitive assay. In this assay a known analyte, (such as a protein of the present invention) is immobilized on a solid substrate. A known amount of antibody specifically reactive, under immunoreactive

conditions, to the protein is added to the sample, and the sample is then contacted with the immobilized protein. In this case, the amount of antibody bound to the immobilized protein is inversely proportional to the amount of protein present in the sample. Again, the amount of immobilized antibody may be detected by detecting either the immobilized fraction of antibody or the fraction of the antibody that remains in solution. Detection may be direct where the antibody is labeled or indirect by the subsequent addition of a labeled moiety that specifically binds to the antibody as described above.

C. Generation of pooled antisera for use in immunoassays

5

10

15

20

25

30

A protein that specifically binds to or that is specifically immunoreactive with an antibody generated against a defined immunogen is determined in an immunoassay. The immunoassay uses a polyclonal antiserum which is raised to a polypeptide of the present invention (i.e., the immunogenic polypeptide). This antiserum is selected to have low crossreactivity against other proteins and any such crossreactivity is removed by immunoabsorbtion prior to use in the immunoassay (e.g., by immunosorbtion of the antisera with a protein of different substrate specificity (e.g., a different enzyme) and/or a protein with the same substrate specificity but of a different form).

In order to produce antisera for use in an immunoassay, a polypeptide of the present invention is isolated as described herein. For example, recombinant protein can be produced in a mammalian or other eukaryotic cell line. An inbred strain of mice is immunized with the protein using a standard adjuvant, such as Freund's adjuvant, and a standard mouse immunization protocol (see Harlow and Lane, above). Alternatively, a synthetic polypeptide derived from the sequences disclosed herein and conjugated to a carrier protein is used as an immunogen. Polyclonal sera are collected and titered against the immunogenic polypeptide in an immunoassay, for example, a solid phase immunoassay with the immunogen immobilized on a solid support. Polyclonal antisera with a titer of 10⁴ or greater are selected and tested for their cross reactivity against polypeptides of different forms or substrate specificity, using a competitive binding immunoassay such as the one described in Harlow and Lane, above, at pages 570-573. Preferably, two or more distinct forms of polypeptides are used in this determination. These distinct types of polypeptides are used as competitors to identify antibodies which are specifically bound by the polypeptide being assayed for. The competitive

- 69 -

polypeptides can be produced as recombinant proteins and isolated using standard molecular biology and protein chemistry techniques as described herein.

Immunoassays in the competitive binding format are used for crossreactivity determinations. For example, the immunogenic polypeptide is immobilized to a solid support. Proteins added to the assay compete with the binding of the antisera to the immobilized antigen. The ability of the above proteins to compete with the binding of the antisera to the immobilized protein is compared to the immunogenic polypeptide. The percent crossreactivity for the above proteins is calculated, using standard calculations. Those antisera with less than 10% crossreactivity with a distinct form of a polypeptide are selected and pooled. The cross-reacting antibodies are then removed from the pooled antisera by immunoabsorbtion with a distinct form of a polypeptide.

The immunoabsorbed and pooled antisera are then used in a competitive binding immunoassay as described herein to compare a second "target" polypeptide to the immunogenic polypeptide. In order to make this comparison, the two polypeptides are each assayed at a wide range of concentrations and the amount of each polypeptide required to inhibit 50% of the binding of the antisera to the immobilized protein is determined using standard techniques. If the amount of the target polypeptide required is less than twice the amount of the immunogenic polypeptide that is required, then the target polypeptide is said to specifically bind to an antibody generated to the immunogenic protein. As a final determination of specificity, the pooled antisera is fully immunosorbed with the immunogenic polypeptide until no binding to the polypeptide used in the immunosorbtion is detectable. The fully immunosorbed antisera is then tested for reactivity with the test polypeptide. If no reactivity is observed, then the test polypeptide is specifically bound by the antisera elicited by the immunogenic protein.

25

30

20

5

10

15

D. Other Assay Formats

In a particularly preferred embodiment, Western blot (immunoblot) analysis is used to detect and quantify the presence of protein of the present invention in the sample. The technique generally comprises separating sample proteins by gel electrophoresis on the basis of molecular weight, transferring the separated proteins to a suitable solid support, (such as a nitrocellulose filter, a nylon filter, or derivatized nylon filter), and incubating the sample with the antibodies that specifically bind a protein of the present invention. The antibodies specifically bind to the protein on the solid support. These

WO 00/09706

antibodies may be directly labeled or alternatively may be subsequently detected using labeled antibodies (e.g., labeled sheep anti-mouse antibodies) that specifically bind to the antibodies.

5

E. Quantification of Proteins.

The proteins of the present invention may be detected and quantified by any of a number of means well known to those of skill in the art. These include analytic biochemical methods such as electrophoresis, capillary electrophoresis, high performance liquid chromatography (HPLC), thin layer chromatography (TLC), hyperdiffusion chromatography, and the like, and various immunological methods such as fluid or gel precipitin reactions, immunodiffusion (single or double), immunoelectrophoresis, radioimmunoassays (RIAs), enzyme-linked immunosorbent assays (ELISAs), immunofluorescent assays, and the like.

15

20

10

F. Reduction of Non-Specific Binding

One of skill will appreciate that it is often desirable to reduce non-specific binding in immunoassays and during analyte purification. Where the assay involves an antigen, antibody, or other capture agent immobilized on a solid substrate, it is desirable to minimize the amount of non-specific binding to the substrate. Means of reducing such non-specific binding are well known to those of skill in the art. Typically, this involves coating the substrate with a proteinaceous composition. In particular, protein compositions such as bovine serum albumin (BSA), nonfat powdered milk, and gelatin are widely used.

25

30

G. Immunoassay Labels

The labeling agent can be, e.g., a monoclonal antibody, a polyclonal antibody, a binding protein or complex, or a polymer such as an affinity matrix, carbohydrate or lipid. Detectable labels suitable for use in the present invention include any composition detectable by spectroscopic, radioisotopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Detection may proceed by any known method, such as immunoblotting, western analysis, gel-mobility shift assays, fluorescent in situ hybridization analysis (FISH), tracking of radioactive or

- 71 -

bioluminescent markers, nuclear magnetic resonance, electron paramagnetic resonance, stopped-flow spectroscopy, column chromatography, capillary electrophoresis, or other methods which track a molecule based upon an alteration in size and/or charge. The particular label or detectable group used in the assay is not a critical aspect of the invention. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and, in general, any label useful in such methods can be applied to the present invention. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Useful labels in the present invention include magnetic beads, fluorescent dyes, radiolabels, enzymes, and colorimetric labels or colored glass or plastic beads, as discussed for nucleic acid labels, above.

5

10

15

20

25

30

The label may be coupled directly or indirectly to the desired component of the assay according to methods well known in the art. As indicated above, a wide variety of labels may be used, with the choice of label depending on the sensitivity required, ease of conjugation of the compound, stability requirements, available instrumentation, and disposal provisions.

Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (e.g., biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (e.g., streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, for example, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody.

The molecules can also be conjugated directly to signal generating compounds, e.g., by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds include fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, etc. Chemiluminescent compounds include luciferin, and 2,3-dihydrophthalazinediones, e.g.,

10

15

25

30

luminol. For a review of various labeling or signal producing systems which may be used, see, U.S. Patent No. 4,391,904, which is incorporated herein by reference.

Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it may be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence, e.g., by microscopy, visual inspection, via photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels may be detected by providing appropriate substrates for the enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels may be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

20 Assays for Compounds that Modulate Enzymatic Activity or Expression

The present invention also provides means for identifying compounds that bind to (e.g., substrates), and/or increase or decrease (i.e., modulate) the enzymatic activity of, catalytically active polypeptides of the present invention. The method comprises contacting a polypeptide of the present invention with a compound whose ability to bind to or modulate enzyme activity is to be determined. The polypeptide employed will have at least 20%, preferably at least 30% or 40%, more preferably at least 50% or 60%, and most preferably at least 70% or 80% of the specific activity of the native, full-length polypeptide of the present invention (e.g., enzyme). Generally, the polypeptide will be present in a range sufficient to determine the effect of the compound, typically about 1 nM to $10~\mu M$. Likewise, the compound will be present in a concentration of from about 1 nM to $10~\mu M$. Those of skill will understand that such factors as enzyme concentration, ligand concentrations (i.e., substrates, products, inhibitors, activators), pH, ionic strength, and temperature will be controlled so as to obtain useful kinetic data

- 73 -

and determine the presence of absence of a compound that binds or modulates polypeptide activity. Methods of measuring enzyme kinetics is well known in the art. See, e.g., Segel, Biochemical Calculations, 2nd ed., John Wiley and Sons, New York (1976).

Although the present invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

10 Example 1

5

This example describes the construction cDNA libraries.

Total RNA Isolation

Total RNA was isolated from corn tissues with TRIzol Reagent (Life Technology Inc. Gaithersburg, MD) using a modification of the guanidine isothiocyanate/acid-phenol 15 procedure described by Chomczynski and Sacchi (Chomczynski, P., and Sacchi, N. Anal. Biochem. 162, 156 (1987)). In brief, plant tissue samples were pulverized in liquid nitrogen before the addition of the TRIzol Reagent, and then were further homogenized with a mortar and pestle. Addition of chloroform followed by centrifugation was conducted for separation of an aqueous phase and an organic phase. 20 The total RNA was recovered by precipitation with isopropyl alcohol from the aqueous phase.

Poly(A) + RNA Isolation

25 The selection of poly(A) + RNA from total RNA was performed using PolyATact system (Promega Corporation. Madison, WI). In brief, biotinylated oligo(dT) primers were used to hybridize to the 3' poly(A) tails on mRNA. The hybrids were captured using streptavidin coupled to paramagnetic particles and a magnetic separation stand. The mRNA was washed at high stringent condition and eluted by RNase-free deionized 30 water.

cDNA Library Construction

- 74 -

cDNA synthesis was performed and unidirectional cDNA libraries were constructed using the SuperScript Plasmid System (Life Technology Inc. Gaithersburg, MD). The first stand of cDNA was synthesized by priming an oligo(dT) primer containing a Not I site. The reaction was catalyzed by SuperScript Reverse Transcriptase II at 45°C. The second strand of cDNA was labeled with alpha-32P-dCTP and a portion of the reaction was analyzed by agarose gel electrophoresis to determine cDNA sizes. cDNA molecules smaller than 500 base pairs and unligated adapters were removed by Sephacryl-S400 chromatography. The selected cDNA molecules were ligated into pSPORT1 vector in between of Not I and Sal I sites.

10

15

20

25

30

5

Example 2

This example describes cDNA sequencing and library subtraction.

Sequencing Template Preparation

Individual colonies were picked and DNA was prepared either by PCR with M13 forward primers and M13 reverse primers, or by plasmid isolation. All the cDNA clones were sequenced using M13 reverse primers.

Q-bot Subtraction Procedure

cDNA libraries subjected to the subtraction procedure were plated out on 22 x 22 cm² agar plate at density of about 3,000 colonies per plate. The plates were incubated in a 37°C incubator for 12-24 hours. Colonies were picked into 384-well plates by a robot colony picker, Q-bot (GENETIX Limited). These plates were incubated overnight at 37°C.

Once sufficient colonies were picked, they were pinned onto 22 x 22 cm² nylon membranes using Q-bot. Each membrane contained 9,216 colonies or 36,864 colonies. These membranes were placed onto agar plate with appropriate antibiotic. The plates were incubated at 37°C for overnight.

After colonies were recovered on the second day, these filters were placed on filter paper prewetted with denaturing solution for four minutes, then were incubated on top of a boiling water bath for additional four minutes. The filters were then placed on filter paper prewetted with neutralizing solution for four minutes. After excess solution was removed by placing the filters on dry filter papers for one minute, the colony side of the filters were place into Proteinase K solution, incubated at 37°C for 40-50 minutes.

The filters were placed on dry filter papers to dry overnight. DNA was then cross-linked to nylon membrane by UV light treatment.

Colony hybridization was conducted as described by Sambrook, J., Fritsch, E.F. and Maniatis, T., (in Molecular Cloning: A laboratory Manual, 2nd Edition). The following probes were used in colony hybridization:

- First strand cDNA from the same tissue as the library was made from to remove the most redundant clones.
- 48-192 most redundant cDNA clones from the same library based on previous sequencing data.
- 10 3. 192 most redundant cDNA clones in the entire corn partial sequence database.

 - 5. cDNA clones derived from rRNA.

The image of the autoradiography was scanned into computer and the signal intensity and cold colony addresses of each colony was analyzed. Re-arraying of cold-colonies from 384 well plates to 96 well plates was conducted using Q-bot.

Example 3

This example describes identification of the gene from a computer homology 20 Gene identities were determined by conducting BLAST (Basic Local search. Alignment Search Tool; Altschul, S. F., et al., (1993) J. Mol. Biol. 215:403-410; see also www.ncbi.nlm.nih.gov/BLAST/) searches under default parameters for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein 25 sequence database, EMBL, and DDBJ databases). The cDNA sequences were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm. The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish, W. and States, D. J. (1993) Nature 30 Genetics 3:266-272) provided by the NCBI. In some cases, the sequencing data from two or more clones containing overlapping segments of DNA were used to construct contiguous DNA sequences.

- 76 -

The above examples are provided to illustrate the invention but not to limit its scope. Other variants of the invention will be readily apparent to one of ordinary skill in the art and are encompassed by the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference.

WHAT IS CLAIMED IS:

- 1. An isolated nucleic acid comprising a member selected from the group consisting of:
- (a) a polynucleotide having at least 80% sequence identity, as determined by the BLAST 2.0 algorithm under default parameters, to a polynucleotide encoding a polypeptide selected from the group consisting of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58
 - (b) a polynucleotide encoding a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58
 - (c) a polynucleotide amplified from a Zea mays nucleic acid library using primers which selectively hybridize, under stringent hybridization conditions, to loci within a polynucleotide selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57
- (d) a polynucleotide which selectively hybridizes, under stringent hybridization conditions and a wash in 2X SSC at 50°C, to a polynucleotide of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57
 - (e) a polynucleotide of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57
- 20 (f) a polynucleotide which is complementary to a polynucleotide of (a), (b), (c), (d), or (e); and
 - (g) a polynucleotide comprising at least 25 contiguous nucleotides from a polynucleotide of (a), (b), (c), (d), (e), or (f).
- 25 2. A recombinant expression cassette, comprising a member of claim 1 operably linked, in sense or anti-sense orientation, to a promoter.
 - 3. A host cell comprising the recombinant expression cassette of claim 2.
- 30 4. A transgenic plant comprising a recombinant expression cassette of claim 2.
 - 5. The transgenic plant of claim 4, wherein the plant is a monocot.

- 6. The transgenic plant of claim 4, wherein the plant is selected from the group consisting of: maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, and millet.
- 5 7. A transgenic seed from the transgenic plant of claim 4.
 - 8. A method of modulating the level of cellulose synthase in a plant cell capable of plant regeneration, comprising:
 - (a) transforming the plant cell with a recombinant expression cassette comprising a cellulose synthase polynucleotide of claim 1 operably linked to a promoter;
 - (b) culturing the transformed plant cell; and

25

30

- (c) inducing expression of said polynucleotide for a time sufficient to modulate the level of cellulose synthase in said transformed plant cell.
- 15 9. The method of claim 8, wherein a plant is regenerated from the transformed plant cell.
- 10. The method of claim 9, wherein the plant is selected from the group consisting of : maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, and millet.
 20 millet.
 - 11. The method of claim 8, wherein the promoter is a tissue-preferred promoter.
 - 12. The method of claim 8, wherein the level of cellulose synthase is increased.
 - 13. The method of claim 8 wherein the cell cycle polynucleotide is amplified from a Zea mays nucleic acid library using primers which selectively hybridize, under stringent hybridization conditions, to loci within a polynucleotide selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57.
 - 14. The method of claim 8 wherein the cell cycle gene is selected from the group consisting of SEQ ID NOS: 1, 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57.

- 15. An isolated protein comprising a member selected from the group consisting of:
 - (a) a polypeptide of at least 20 contiguous amino acids from a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58
 - (b) a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58
 - (c) a polypeptide having at least 80% sequence identity to, and having at least one linear epitope in common with, a polypeptide of SEQ ID NOS: 2, 6, 10, 14, 18, 22, 26, 30, 34, 38, 42, 46, 50, 54, and 58, wherein said sequence identity is determined using BLAST 2.0 under default parameters; and,
- 10 (d) a polypeptide encoded by a member of claim 1.

- 1 -

SEQUENCE LISTING

<110> Pioneer Hi-Bred International, Inc.

<120> Maize Cellulose Synthases and Uses Thereof

<130> 0864-PCT

<150> 60/096,822

<151> August 17, 1998

<160> 60

<170> FastSEQ for Windows Version 3.0

<210> 1

<211> 3568

<212> DNA

<213> Zea mays

<220>

<221> CDS

<222> (63)...(3237)

<400> 1

gtcgacccac	gcgtccggag ctcgtcg	gtca teegeegega tggega	gcca gggccgaagc 60
cc atg gac Met Asp	cag cgg aac ggc ca Gln Arg Asn Gly Gl	ag gtg tgc cag att tgc ln Val Cys Gln Ile Cys	ggc gac gac 107 Gly Asp Asp
1	5	10	15

gtg ggg cgc aac ccc gac ggg gag cct ttc gtg gcc tgc aac gag tgc Val Gly Arg Asn Pro Asp Gly Glu Pro Phe Val Ala Cys Asn Glu Cys
20 25 30

gcc ttc ccc atc tgc cgg gac tgc tac gag tac gag cgc cgc gag ggc 203
Ala Phe Pro Ile Cys Arg Asp Cys Tyr Glu Tyr Glu Arg Arg Glu Gly
35

acg cag aac tgc ccc cag tgc aag acc cgc ttc aag cgc ttc aag ggg

Thr Gln Asn Cys Pro Gln Cys Lys Thr Arg Phe Lys Arg Phe Lys Gly

50

55

60

tgc gcg cgc gtg ccc ggg gac gag gag gag gac ggc gtc gac gac ctg

Cys Ala Arg Val Pro Gly Asp Glu Glu Glu Asp Gly Val Asp Asp Leu

65

gag aac gag ttc aac tgg agc gac aag cac gac tcc cag tac ctc gcc
Glu Asn Glu Phe Asn Trp Ser Asp Lys His Asp Ser Gln Tyr Leu Ala
80
85
90
95

gag too atg oto cac goo cac atg ago tac ggo cgc ggc gcc gac oto 395 Glu Ser Met Leu His Ala His Met Ser Tyr Gly Arg Gly Ala Asp Leu 100 105 110

gac ggc gtg ccg cag cca ttc cac ccc atc ccc aat gtt ccc ctc ctc 443

WO 00/09706

- 2 -

									•							
Ası	Gl;	y Va	l Pro	o Glr 5	n Pro	Phe	e Hi≀	120		e Pro	o Ası	n Val	129		u Leu	
acc Thr	aa Ası	gg n Gl	A GTI	g ato Met	gto : Val	gat L Asp	gao Ası 135	o Ile	e Pro	g cco	g gad D Asp	cag Glr 140	His	gc Ala	c ctt a Leu	491
gtg Val	Pro 145) se	g tto r Phe	gtg Val	ggt Gly	ggc Gl _y 150	∖ GT ²	g ggg	j aag ⁄ Lys	g agg	g att g Ile 155	His	ect Pro	: cto	c ccg	539
tac Tyr 160	ALS	gai Asj	cco Pro	aac Asn	ctt Leu 165	Pro	gto Val	g caa Gln	ccg Pro	agg Arg	, Ser	atg Met	gac	cct Pro	tcc Ser 175	587
aag Lys	gat Asp	cto Lev	c gcc ı Ala	gca Ala 180	Tyr	Gly	tac Tyr	Gly Ggg	ago Ser 185	Val	gca Ala	tgg Trp	aag Lys	gag Glu 190	agg Arg	635
atg Met	gag Glu	ago Ser	tgg Trp 195	rys	cag Gln	aag Lys	cag Gln	gag Glu 200	Arg	atg Met	cac His	cag Gln	acg Thr 205	agg Arg	aac Asn	683
gat Asp	ggc	ggc Gly 210	ggc Gly	gat Asp	gat Asp	ggt Gly	gat Asp 215	gat Asp	gca Ala	gat Asp	cta Leu	cca Pro 220	cta Leu	atg Met	gat Asp	731
gaa Glu	gct Ala 225	aga Arg	cag Gln	cca Pro	ttg Leu	tcc Ser 230	aga Arg	aag Lys	atc Ile	ccg Pro	ctt Leu 235	cct Pro	tca Ser	agc Ser	caa Gln	779
atc Ile 240	aac Asn	ccc	tat Tyr	agg Arg	atg Met 245	att Ile	ata Ile	ata Ile	att Ile	cgg Arg 250	cta Leu	gtg Val	gtt Val	ttg Leu	tgt Cys 255	827
ttc Phe	ttc Phe	ttc Phe	cac His	tac Tyr 260	cga Arg	gtg Val	atg Met	cat His	ccg Pro 265	gtg Val	cct Pro	gat Asp	gca Ala	ttt Phe 270	gct Ala	875
tta Leu	tgg Trp	ctc Leu	ata Ile 275	tct Ser	gtg Val	atc Ile	tgt Cys	gaa Glu 280	att Ile	tgg Trp	ttt Phe	Ala	atg Met 285	tct Ser	tgg Trp	923
att Ile	ctt Leu	gac Asp 290	cag Gln	ttt Phe	cca Pro	aag Lys	tgg Trp 295	ttt Phe	cct Pro	atc Ile	gag Glu	agg Arg 300	gaa Glu	acc Thr	tat Tyr	971
Leu .	gac Asp 305	cgg Arg	ctg Leu	agt Ser	Leu .	agg Arg 310	ttt Phe	gac Asp	aag Lys	Glu	999 Gly 315	cat His	cct Pro	tct Ser	caa Gln	1019
ctc (Leu / 320	gcc Ala	cct Pro	gtt Val	Asp .	ttc Phe: 325	ttt Phe	gtc Val	agt Ser	Thr	gtt Val 330	gat Asp	ccc Pro	ttg Leu	aag Lys	gaa Glu 335	1067
ect (cca Pro	ttg Leu	Val	act of the last of	gct a Ala a	aat Asn	act Thr	Val :	cta Leu 345	tct Ser	atc Ile :	ctt (Leu :	Ser '	gtg Val 350	gat Asp	1115

1	tat Tyr	CCa Pro	a gtt val	gat L Asp 359	э гуу	g gtt s Val	tca Ser	tgc Cys	tac Tyr 360	· Val	tct Ser	gat Asp	gat Asp	ggt Gl ₃ 365	/ Ala	gcc Ala	1163
î	atg Met	Leu	aca Thi	Pne	gaa Glu	a gca ı Ala	ttg Leu	Ser 375	Glu	aca Thr	Ser	gaa Glu	ttt Phe 380	Ala	aag Lys	g aaa S Lys	1211
1	rp gg	gtt Val 385	. Pro	tto Phe	tgo Cys	aaa Lys	aga Arg 390	Tyr	agc Ser	ctt Leu	gag Glu	Pro 395	Arg	gct	cca Pro	gag Glu	1259
1	rp Grp	Tyr	tto Phe	cas Glr	cag Gln	aag Lys 405	Ile	gac	tac Tyr	ctg Leu	aaa Lys 410	Asp	aag Lys	gtg Val	gcg	cca Pro 415	1307
A	ac	ttt Phe	gtt Val	aga Arg	gaa Glu 420	Arg	aga Arg	gca Ala	atg Met	aag Lys 425	Arg	gag Glu	tat Tyr	gag Glu	gaa Glu 430	ttc Phe	1355
a L	ys	gtc Val	aga Arg	ato Ile 435	Asn	gcc Ala	ttg Leu	gtt Val	gct Ala 440	aaa Lys	gcc Ala	caa Gln	aag Lys	gtt Val 445	cct Pro	gag Glu	1403
g	aa lu	gga Gly	tgg Trp 450	aca Thr	atg Met	cag Gln	gat Asp	gga Gly 455	act Thr	cca Pro	tgg Trp	ccc Pro	gga Gly 460	aat Asn	aat Asn	gtc Val	1451
A	gt rg	gat Asp 465	cat His	cct Pro	gga Gly	atg Met	att Ile 470	cag Gln	gtt Val	ttc Phe	ctt Leu	ggt Gly 475	caa Gln	agt Ser	ggt Gly	ggc Gly	1499
Н	at is 80	gat Asp	gtg Val	gaa Glu	gga Gly	aat Asn 485	gag Glu	ctg Leu	cct Pro	cga Arg	ttg Leu 490	gtt Val	tat Tyr	gtt Val	tca Ser	aga Arg 495	1547
g G	aa lu	aaa Lys	cgg Arg	cca Pro	ggc Gly 500	tac Tyr	aac Asn	cat His	cac His	aag Lys 505	aag Lys	gct Ala	ggt Gly	gct Ala	atg Met 510	aat Asn	1595
g A	ca la	ttg Leu	gtc Val	cga Arg 515	gtc Val	tct Ser	gct Ala	gta Val	cta Leu 520	act Thr	aat Asn	gct Ala	cct Pro	tat Tyr 525	ttg Leu	ctg Leu	1643
aa As	ac sn	ttg Leu	gat Asp 530	tgt Cys	gat Asp	cac His	tat Tyr	atc Ile 535	aat Asn	aat Asn	agt Ser	aag Lys	gct Ala 540	ata Ile	aag Lys	gaa Glu	1691
go Al	La :	atg Met 545	tgt Cys	ttt Phe	atg Met	atg Met	gat Asp 550	cct Pro	ttg Leu	ctt Leu	gga Gly	aag Lys 555	aaa Lys	gtt Val	tgc Cys	tat Tyr	1739
gt Va 56	11	cag Gln	ttt Phe	cct Pro	caa Gln	aga Arg 565	ttt Phe	gat Asp	gly ggg	att Ile	gat Asp 570	cgc Arg	cat His	gat Asp	cga Arg	tat Tyr 575	1787
go	et i	aac	aga	aat	gtt	gtc	ttt	ttc	gat	atc	aac	atg	aaa	ggt	ttg	gat	1835

Al	a As	n Ar	g As	n Va 58	1 Va:	l Pho	e Pho	e As	p Ile 58	e Ası 5	n Me	t Ly	s Gl	y Le 59	u Asp O	
G1	t at y Il	c ca e Gl	n Gl 59	y Pr	a att	t tat	gtg Val	g ggt L Gl ₃ 600	y Thi	gga Gly	a tg / Cy	t gte s Vai	tt l Ph	e Ar	a agg g Arg	1883
Ca Gli	g gc	a tt a Le 61	uly	t gg r Gl	c tac y Tyr	gat Asp	gct Ala 615	Pro	c aaa D Lys	a aca	a aag	g aag s Lys 620	Pro	a cc	a tca Ser	1931
aga Arg	a act	r cy	c aa s As:	c tgo	tgg Trp	p cca Pro 630	Lys	tgg Trp	tgc Cys	att Ile	tg: Cy:	з Суя	tgo Cys	tgi G Cys	ttt Phe	1979
ggt Gl _} 640	ASI	ag n Ar	g aaq g Ly:	g aco	aag Lys 645	Lys	aag Lys	acc Thr	aag Lys	acc Thr 650	Sei	aaa Lys	cct Pro	aaa Lys	ttt Phe 655	2027
gag Glu	aag Lys	g ata	a aag e Lys	aaa Lys 660	Leu	ttt Phe	aag Lys	aaa Lys	aag Lys 665	Glu	aat Asr	caa Gln	gcc	Pro 670	gca Ala	2075
tat Tyr	gct Ala	ctt Let	ggt 1 Gly 675	GIU	att Ile	gat Asp	gaa Glu	gcc Ala 680	gct Ala	cca Pro	gga Gly	gct Ala	gaa Glu 685	Asn	gaa Glu	2123
aag Lys	gct Ala	agt Ser 690	. 116	gta Val	aat Asn	caa Gln	cag Gln 695	aag Lys	ttg Leu	gaa Glu	aag Lys	aaa Lys 700	ttt Phe	ggc	cag Gln	2171
tct Ser	tca Ser 705	gtt Val	ttt. Phe	gtt Val	gca Ala	tcc Ser 710	aca Thr	ctt Leu	ctt Leu	gag Glu	aat Asn 715	ggt Gly	gga Gly	acc Thr	ctg Leu	2219
aag Lys 720	agt Ser	gcc	agt Ser	cca Pro	gct Ala 725	tct Ser	ctt Leu	ctg Leu	aag Lys	gaa Glu 730	gct Ala	ata Ile	cat His	gtc Val	atc Ile 735	2267
agt Ser	tgt Cys	gga Gly	tat Tyr	gaa Glu 740	gac Asp	aaa Lys	aca Thr	ggc Gly	tgg Trp 745	gga Gly	aaa Lys	gat Asp	att Ile	ggt Gly 750	tgg Trp	2315
att Ile	tat Tyr	gga Gly	tca Ser 755	gtc Val	aca Thr	gaa Glu	Asp	att Ile 760	ctt Leu	act Thr	glà aaa	ttt Phe	aag Lys 765	atg Met	cac His	2363
tgc Cys	cat His	ggt Gly 770	tgg Trp	cgg Arg	tca Ser	Ile	tac Tyr 775	tgc Cys	ata Ile	cct Pro	aaa Lys	cgg Arg 780	gcc Ala	gcc Ala	ttc Phe	2411
aaa Lys	ggt Gly 785	tcc Ser	gca Ala	cct Pro	ctc Leu	aat Asn 790	ctt Leu	tcc Ser	gat Asp	Arg	ttt Phe 795	cac His	cag Gln	gtt Val	ctt Leu	2459
cgg Arg 800	tgg Trp	gct Ala	ctt Leu	Gly	tca : Ser : 805	att (gaa Glu	att Ile	Leu	ttc Phe 810	agc Ser	aac Asn	cac His	tgc Cys	cct Pro 815	2507

ctc Leu	tgg Trp	tat Tyr	. Gly	tat Tyr 820	GIA	ggt Gly	gga Gly	a cta / Leu	aag Lys 825	5 Ph	c ct	g gaa u Glu	a agg	tti Phe 830	tcg Ser	2555
-11-	110	ASII	835	116	vai	Tyr	Pro	840	The	Se	r Ile	e Pro	845	Let	gee Ala	2603
tat Tyr	tgc Cys	aca Thr 850	neu	Pro	gcc Ala	atc Ile	tgo Cys 855	Leu	ctg Leu	aca Thi	Gly	g aaa / Lys 860	Phe	ato Ile	acg Thr	2651
FIO	865	rea	Asn	Asn	Vai	Ala 870	Ser	Leu	Trp	Phe	875		Leu	Phe	Ile	2699
880	110	1116	ALG	III	885	iie	Leu	Giu	Met	Arg 890	Trp	g agt Ser	Gly	Val	Gly 895	2747
116	Asp	ASD	irp	900	Arg	Asn	Glu	Gln	Phe 905	Trp	Val	att Ile	Gly	Gly 910	Val	2795
501			915	PHE	Ala	vaı	Pne	920	Gly	Leu	Leu	aag Lys	Val 925	Ile	Ala	2843
O.L.	Vai	930	****	ser	Pne	rnr	935	Thr	Ser	Lys	Gly	gga Gly 940	Asp	Asp	Glu	2891
GIU .	945	ser	GIU	Leu	Tyr	Thr 950	Phe	Lys	Trp	Thr	Thr 955	ctt Leu	Leu	Ile	Pro	2939
eeg a Pro 9	aca Thr	acc Thr	ctg Leu	Leu	cta Leu 965	ctg Leu	aac Asn	ttc Phe	att Ile	gga Gly 970	gtg Val	gta Val	gct Ala	ggc	atc Ile 975	2987
tcc a	aat :	gcg . Ala	TIE .	aac Asn 980	aac Asn	gga Gly	tat Tyr	Glu	tca Ser 985	tgg Trp	ggc Gly	ccc Pro	Leu	ttc Phe 990	gjå aaa	3035
aag d Lys I	etc Leu	ttc Phe	Phe i	gca Ala	ttt ' Phe '	tgg (Irp '	Val	atc (Ile '	gtc Val	cat His	ctt Leu	Tyr	ccg Pro 1005	ttc Phe	ctc Leu	3083
aag g Lys G	TA	ctg g Leu V L010	gtt g /al (aly i	agg (Arg (31n i	aac Asn 7	agg a Arg :	acg Thr	cca Pro	acg Thr	att i Ile 1 1020	gtc a Val :	att (gtc Val	3131
tgg t Trp S	cc a er 1 025	itc o	etc d Leu I	etg g Leu 1	Ala S	cg a Ser 1	atc i	ttc t Phe S	er i	Leu	ctt Leu 1035	Trp V	gtc (/al /	egg a	atc Ile	3179
gac c	cg t	tc c	tt g	geg a	ag g	gat c	gat o	ggt d	ecc (ctg	ttg	gag g	gag t	gt	ggt	3227

- 6 -

As ₁	p Pro 40	o Ph	e Le	u Al	a Ly:	s As _j 45	p As	p Gl	y Pr	o Le		u Gl	u Gl	u Cy	s Gly 1055	
Ct:	g ga u Asj	t tg	caa s	acta	ggag	gt c	agca	cgtg	g ac	ttcc	ccgt	cag	tgtg	tgg		3277
gtg	gcago ggtgg gcaao	tgg ctt	tggt	caagg cgggd cagt1	egg t egg t	gaag ctcag ccag	ggg; gcct; gaa!	aa aa cg ta tn ta	aaaag gagtg actac	gtaci gcaai gggai	t tgi L ati	eatti eggge	tctt	ccg	tgtccct ttccatg Jaggttg caatcaa	3397
	<	210: 211: 212:		59												
M - 4		400>														
Met 1	: Asp	Gln	Arg	Asn 5	Gly	Gln	Val	. Суғ	Gln 10	Ile	Сув	Gly	/ Asp	Asp	Val	
Gly	Arg	Asn	Pro	Asp	Gly	Glu	Pro			Ala	Cys	Asr		Cys	Ala	
Phe	Pro	Ile 35		Arg	Asp	Cys	Тут 40	25 Glu	Tyr	Glu	Arg	Arg	30 Glu	Gly	Thr	
Gln	Asn 50	Суз	Pro	Gln	Cys	Lys 55	Thr	Arg	, Phe	Lys		Phe	Lys	Gly	Cys	
Ala		Val	Pro	Gly	Asp		Glu	Glu	Asp	Gly	60 Val	Asp	Asp	Leu	Glu	
0.5					70					75					80 Glu	
				85					90					9.5		
			TOO					105					Asp			
GIY	vai	Pro 115	Gln	Pro	Phe	His	Pro 120	Ile	Pro	Asn	Val	Pro 125	Leu	Leu	Thr	
Asn	Gly 130	Gln	Met	Val	Asp	Asp 135	Ile	Pro	Pro	Asp		His	Ala	Leu	Val	
Pro		Phe	Val	Gly	Gly		Gly	Lys	Arg	Ile	140 His	Pro	Leu	Pro	Tyr	
145					150					155			Pro		160	
				165					170					175		
Asp	rea	Ala	180	Tyr	GIY	Tyr	Gly	Ser 185	Val	Ala	Trp	Lys	Glu 190	Arg	Met	
		195					200					205	Arg			
Gly	Gly 210	Gly	qaA	Asp	Gly	Asp 215	Asp	Ala	Asp	Leu	Pro 220	Leu	Met	Asp	Glu	
Ala	Arg	Gln	Pro	Leu	Ser		Lys	Ile	Pro		Pro	Ser	Ser	Gln	Ile	
225 Asn	Pro	Tyr	Arg	Met	230 Ile	Ile	Ile	Ile	Ara	235 Leu	Val	Val	Leu	Cve	240 Phe	
				245					250					255		
			260					265					Phe 270			
Trp	Leu	Ile 275	Ser	Val	Ile	Cys	Glu 280	Ile	Trp	Phe	Ala		Ser	Trp	Ile	
Leu	Asp 290		Phe	Pro	Lys	Trp 295		Pro	Ile	Glu	Arg 300	285 Glu	Thr	Tyr	Leu	
Asp	Arg	Leu	Ser	Leu	Arg		Asp	Lys	Glu	Gly	His	Pro	Ser	Gln	Leu	

- 7 -

305 310 315 Ala Pro Val Asp Phe Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro 325 330 Pro Leu Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr 345 Pro Val Asp Lys Val Ser Cys Tyr Val Ser Asp Asp Gly Ala Ala Met 360 Leu Thr Phe Glu Ala Leu Ser Glu Thr Ser Glu Phe Ala Lys Lys Trp 375 380 Val Pro Phe Cys Lys Arg Tyr Ser Leu Glu Pro Arg Ala Pro Glu Trp 390 395 Tyr Phe Gln Gln Lys Ile Asp Tyr Leu Lys Asp Lys Val Ala Pro Asn 405 410 Phe Val Arg Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys 420 · 425 Val Arg Ile Asn Ala Leu Val Ala Lys Ala Gln Lys Val Pro Glu Glu 440 Gly Trp Thr Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val Arg 455 460 Asp His Pro Gly Met Ile Gln Val Phe Leu Gly Gln Ser Gly Gly His 470 475 Asp Val Glu Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu 485 490 Lys Arg Pro Gly Tyr Asn His His Lys Lys Ala Gly Ala Met Asn Ala 500 505 Leu Val Arg Val Ser Ala Val Leu Thr Asn Ala Pro Tyr Leu Leu Asn 520 Leu Asp Cys Asp His Tyr Ile Asn Asn Ser Lys Ala Ile Lys Glu Ala 535 Met Cys Phe Met Met Asp Pro Leu Leu Gly Lys Lys Val Cys Tyr Val 550 555 Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg His Asp Arg Tyr Ala 570 Asn Arg Asn Val Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly 580 585 Ile Gln Gly Pro Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Arg Gln 600 Ala Leu Tyr Gly Tyr Asp Ala Pro Lys Thr Lys Lys Pro Pro Ser Arg 615 620 Thr Cys Asn Cys Trp Pro Lys Trp Cys Ile Cys Cys Cys Phe Gly 630 635 Asn Arg Lys Thr Lys Lys Thr Lys Thr Ser Lys Pro Lys Phe Glu 645 650 Lys Ile Lys Lys Leu Phe Lys Lys Lys Glu Asn Gln Ala Pro Ala Tyr 665 Ala Leu Gly Glu Ile Asp Glu Ala Ala Pro Gly Ala Glu Asn Glu Lys 680 Ala Ser Ile Val Asn Gln Gln Lys Leu Glu Lys Lys Phe Gly Gln Ser 695 Ser Val Phe Val Ala Ser Thr Leu Leu Glu Asn Gly Gly Thr Leu Lys 710 Ser Ala Ser Pro Ala Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser 730 Cys Gly Tyr Glu Asp Lys Thr Gly Trp Gly Lys Asp Ile Gly Trp Ile 745 Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Cys 760 His Gly Trp Arg Ser Ile Tyr Cys Ile Pro Lys Arg Ala Ala Phe Lys

- 8 -

```
775
                                        780
Gly Ser Ala Pro Leu Asn Leu Ser Asp Arg Phe His Gln Val Leu Arg
                 790 795
Trp Ala Leu Gly Ser Ile Glu Ile Leu Phe Ser Asn His Cys Pro Leu
              805
                   810
Trp Tyr Gly Tyr Gly Gly Gly Leu Lys Phe Leu Glu Arg Phe Ser Tyr
           820
                            825
Ile Asn Ser Ile Val Tyr Pro Trp Thr Ser Ile Pro Leu Leu Ala Tyr
                         840
Cys Thr Leu Pro Ala Ile Cys Leu Leu Thr Gly Lys Phe Ile Thr Pro
                     855
Glu Leu Asn Asn Val Ala Ser Leu Trp Phe Met Ser Leu Phe Ile Cys
                          875
                 870.
Ile Phe Ala Thr Ser Ile Leu Glu Met Arg Trp Ser Gly Val Gly Ile
              885
                                890
Asp Asp Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Val Ser
          900
                            905
Ser His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Ile Ala Gly
                        920
Val Asp Thr Ser Phe Thr Val Thr Ser Lys Gly Gly Asp Asp Glu Glu
                  935
                                       940
Phe Ser Glu Leu Tyr Thr Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro
                950
                                   955
Thr Thr Leu Leu Leu Leu Asn Phe Ile Gly Val Val Ala Gly Ile Ser
             965
                                970
Asn Ala Ile Asn Asn Gly Tyr Glu Ser Trp Gly Pro Leu Phe Gly Lys
          980
                            985
Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys
                        1000
Gly Leu Val Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp
   1010 1015
                                       1020
Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Ile Asp
                1030
                                   1035
Pro Phe Leu Ala Lys Asp Asp Gly Pro Leu Leu Glu Glu Cys Gly Leu
              1045
                                1050
Asp Cys Asn
     <210> 3
```

<211> 25

<212> DNA

<213> Zea mays

<400> 3

atggaccagc ggaacggcca ggtgt

<210> 4

<211> 25

<212> DNA

<213> Zea mays

<400> 4

ctagttgcaa tccagaccac actcc

25

<210> 5

<211> 3773

- 9 -

<212> DNA <213> Zea mays <220> <221> CDS <222> (338)...(3566) <400> 5 gtcgacccac gcgtccgcta ggatcaaaac cgtctcgccg ctgcaataat cttttgtcaa 60 ttettaatee etegegtega cagegacage ggaaccaact caegttgeeg eggetteete 120 categgtgcg gtgccctgtc cttttctctc gtccctcctc cccccgtata gttaagcccc 180 geceegetac tactactact ageageagea gegetetege agegggagat geggtgttga 240 tecgtgeece geteggatet egggaetggt geeggetetg eccaggeece aggetecagg 300 ccagetecet egacgtttet eggegagete gettgee atg gag gge gae geg gae 355 Met Glu Gly Asp Ala Asp ggc gtg aag tcg ggg agg cgc ggt ggc gga cag gtg tgc cag atc tgc 403 Gly Val Lys Ser Gly Arg Gly Gly Gly Gln Val Cys Gln Ile Cys ggc gac ggc gtg ggc acc acg gcg gag ggg gac gtc ttc gcc gcc tgc 451 Gly Asp Gly Val Gly Thr Thr Ala Glu Gly Asp Val Phe Ala Ala Cys gac gtc tgc ggg ttt ccg gtg tgc cgc ccc tgc tac gag tac gag cgc 499 Asp Val Cys Gly Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg 40 aag gac ggc acg cag gcg tgc ccc cag tgc aag acc aag tac aag cgc 547 Lys Asp Gly Thr Gln Ala Cys Pro Gln Cys Lys Thr Lys Tyr Lys Arg 55 cac aag ggg agc ccg gcg atc cgt ggg gag gaa gga gac gac act gat 595 His Lys Gly Ser Pro Ala Ile Arg Gly Glu Glu Gly Asp Asp Thr Asp 75 gcc gat agc gac ttc aat tac ctt gca tct ggc aat gag gac cag aag 643 Ala Asp Ser Asp Phe Asn Tyr Leu Ala Ser Gly Asn Glu Asp Gln Lys 90 cag aag att gcc gac aga atg cgc agc tgg cgc atg aac gtt ggg ggc 691 Gln Lys Ile Ala Asp Arg Met Arg Ser Trp Arg Met Asn Val Gly Gly age ggg gat gtt ggt ege eec aag tat gae agt gge gag ate ggg ett 739 Ser Gly Asp Val Gly Arg Pro Lys Tyr Asp Ser Gly Glu Ile Gly Leu 120 125 acc aag tat gac agt ggc gag att cct cgg gga tac atc cca tca gtc 787 Thr Lys Tyr Asp Ser Gly Glu Ile Pro Arg Gly Tyr Ile Pro Ser Val 140 act aac agc cag atc tca gga gaa atc cct ggt gct tcc cct gac cat 835 Thr Asn Ser Gln Ile Ser Gly Glu Ile Pro Gly Ala Ser Pro Asp His 155 cat atg atg tcc cca act ggg aac att ggc aag cgt gct cca ttt ccc 883

- 10 -

His	Met	: Met	Se: 17(Pro	Thr	Gly	' Asn	11e		/ Lys	Arg	J Ala	180		e Pro	
tat Tyr	gtg Val	aad Asr 185	His	tcg Ser	p cca	aat Asn	ccg Pro	Ser	agg Arg	gag Glu	tto Phe	tct Ser 195	Gly	ago Sei	att : Ile	931
ggg Gly	Asn 200	ı Val	gco Ala	tgg Trp	aaa Lys	gag Glu 205	Arg	gtt Val	gat Asp	ggc	tgg Trp 210	Lys	ato Met	aag Lys	g cag Gln	97 9
gac Asp 215	Lys	gly gag	acg Thr	att Ile	Pro	Met	acg Thr	aat Asn	ggc Gly	aca Thr 225	Ser	att : Ile	gct	ccc Pro	tct Ser 230	1027
gag Glu	ggt	cgg Arg	ggt Gly	gtt Val 235	Gly	gat Asp	att Ile	gat Asp	gca Ala 240	Ser	act Thr	gat Asp	tac	aac Asn 245	atg Met	1075
gaa Glu	gat Asp	gcc Ala	tta Leu 250	Leu	aac Asn	gac Asp	gaa Glu	act Thr 255	cga Arg	cag Gln	cct Pro	cta Leu	tct Ser 260	Arg	aaa Lys	1123
gtt Val	cca Pro	ctt Leu 265	Pro	tcc Ser	tcc Ser	agg Arg	ata Ile 270	aat Asn	cca Pro	tac Tyr	agg Arg	atg Met 275	gtc Val	att Ile	gtg Val	1171
ctg Leu	cga Arg 280	ttg Leu	att Ile	gtt Val	cta Leu	agc Ser 285	atc Ile	ttc Phe	ttg Leu	cac His	tac Tyr 290	cgt Arg	atc Ile	aca Thr	aat Asn	1219
cct Pro 295	gtg Val	cgc Arg	aat Asn	gca Ala	tac Tyr 300	cca Pro	tta Leu	tgg Trp	ctt Leu	cta Leu 305	tct Ser	gtt Val	ata Ile	tgt Cys	gag Glu 310	1267
atc Ile	tgg Trp	ttt Phe	gct Ala	ctt Leu 315	tcg Ser	tgg Trp	ata Ile	ttg Leu	gat Asp 320	cag Gln	ttc Phe	cct Pro	aag Lys	tgg Trp 325	ttt Phe	1315
cca Pro	atc Ile	aac Asn	cgg Arg 330	gag Glu	acg Thr	tac Tyr	ctt Leu	gat Asp 335	agg Arg	ctg Leu	gca Ala	tta Leu	agg Arg 340	tat Tyr	gac Asp	1363
cgg Arg	gaa Glu	ggt Gly 345	gag Glu	cca Pro	tct Ser	cag Gln	ttg Leu 350	gct Ala	gct Ala	gtt Val	gac Asp	att Ile 355	ttc Phe	gtc Val	agt Ser	1411
aca Thr	gtc Val 360	gac Asp	cca Pro	atg Met	aag Lys	gag Glu 365	cct Pro	cct Pro	ctt Leu	gtc Val	act Thr 370	gcc Ala	aat Asn	acc Thr	gtg Val	1459
cta Leu 375	tcc Ser	att Ile	ctt Leu	gct Ala	gtg Val 380	gat Asp	tac Tyr	cct Pro	Val	gat Asp 385	aag Lys	gtc Val	tct Ser	tgc Cys	tat Tyr 390	1507
gta Val	tct Ser	gat Asp	gat Asp	gga Gly 395	gct Ala	gcg Ala	atg Met	Leu	aca Thr 400	ttt Phe	gat Asp	gca Ala	cta Leu	gct Ala 405	gag Glu	1555

act Thi	tca Ser	gaq Gli	y ttt 1 Phe 410	A A L &	aga Arg	aaa J Lys	tgg Trp	gta Val	l Pro	ttt Phe	gtt Val	t aaq l Lys	g aag B Lys 420	з Ту	c aac r Asn	1603
att Ile	gaa Glu	Pro	Arg	gct Ala	cet Pro	gaa Glu	tgg Trp 430	Туг	ttc Phe	tco Ser	cag Glr	aaa Lys 435	: Ile	ga Asj	t tac o Tyr	1651
ttg Leu	aag Lys 440	Asp	aaa Lys	gtg Val	caç His	Pro 445	Ser	ttt Phe	gtt Val	aas Lys	gac Asp 450	Arg	cgg Arg	gco Ala	atg Met	1699
aag Lys 455	Arg	gaa Glu	tat Tyr	gaa Glu	gaa Glu 460	Phe	aaa Lys	gtt Val	agg Arg	gta Val 465	Asn	ggc	ctt Leu	gtt Val	gct Ala 470	1747
aag Lys	gca Ala	cag Gln	aaa Lys	gtt Val 475	Pro	gag Glu	gaa Glu	gga Gly	tgg Trp 480	atc Ile	atg Met	caa Gln	gat Asp	ggc Gly 485	aca Thr	1795
cca Pro	tgg Trp	cca Pro	gga Gly 490	aac Asn	aat Asn	acc Thr	mgg Xaa	gac Asp 495	cat His	cct Pro	gga Gly	atg Met	att Ile 500	cag Gln	gtt Val	1843
ttc Phe	ctt Leu	ggt Gly 505	cac His	agt Ser	ggt Gly	ggc Gly	ctt Leu 510	gat Asp	act Thr	gag Glu	ggc	aat Asn 515	gag Glu	cta Leu	ccc	1891
cgt Arg	ttg Leu 520	gtc Val	tat Tyr	gtt Val	tct Ser	cgt Arg 525	gaa Glu	aag Lys	cgt Arg	cct Pro	gga Gly 530	ttc Phe	cag Gln	cat His	cac His	1939
aag Lys 535	aaa Lys	gct Ala	ggt Gly	gcc Ala	atg Met 540	aat Asn	gct Ala	ctt Leu	gtt Val	cgt Arg 545	gtc Val	tca Ser	gct Ala	gtg Val	ctt Leu 550	1987
acc Thr	aat Asn	gga Gly	caa Gln	tac Tyr 555	atg Met	ttg Leu	aat Asn	ctt Leu	gat Asp 560	tgt Cys	gat Asp	cac His	tac Tyr	att Ile 565	aac Asn	2035
aac Asn	agt Ser	aag Lys	gct Ala 570	ctc Leu	agg Arg	gaa Glu	Ala	atg Met 575	tgc Cys	ttc Phe	ctt Leu	atg Met	gac Asp 580	cct Pro	aac Asn	2083
cta Leu	gga Gly	agg Arg 585	agt Ser	gtc Val	tgc Cys	Tyr	gtc Val 590	cag Gln	ttt Phe	ccc Pro	cag Gln	aga Arg 595	ttc Phe	gat Asp	ggc Gly	2131
att Ile	gac Asp 600	agg Arg	aat Asn	gat Asp	Arg	tat Tyr 605	gcc Ala	aac Asn	agg Arg	Asn	acc Thr 610	gtg Val	ttt Phe	ttc Phe	gat Asp	2179
att Ile 615	aac Asn :	ttg Leu	aga Arg	Gly	ctt Leu 620	gat (Asp (ggc (Gly	atc Ile	Gln	gga Gly 625	cca Pro	gtt Val	tat Tyr	gtc Val	gga Gly 630	2227
act	ggc	tgt	gtt	ttc	aac	cga i	aca g	gct	cta	tat	ggt	tat	gag	ccc	cca	2275

- 12 -

								_								
Thr	Gly	Cys	Val	Phe 635		Arg	Thr	Ala	Leu 640		Gly	Tyr	Glu	Pro 645	Pro	
att Ile	aag Lys	cag Gln	aag Lys 650	Lys	ggt Gly	ggt Gly	ttc Phe	ttg Leu 655	Ser	tca Ser	cta Leu	tgt Cys	ggc Gly 660	Gly	agg Arg	2323
aag Lys	aag Lys	gca Ala 665	Ser	aaa Lys	tca Ser	aag Lys	aag Lys 670	Gly	tcg Ser	gac Asp	aag Lys	aag Lys 675	Lys	tcg Ser	cag Gln	2371
aag Lys	cat His 680	Val	gac Asp	agt Ser	tct Ser	gtg Val 685	cca Pro	gta Val	ttc Phe	aac Asn	ctt Leu 690	gaa Glu	gat Asp	ata Ile	gag Glu	2419
gag Glu 695	gga Gly	gtt Val	gaa Glu	ggc	gct Ala 700	gga Gly	ttt Phe	gac Asp	gac Asp	gag Glu 705	aaa Lys	tca Ser	ctt Leu	ctt Leu	atg Met 710	2467
tct Ser	caa Gln	atg Met	agc Ser	ctg Leu 715	gag Glu	aag Lys	aga Arg	ttt Phe	ggc Gly 720	cag Gln	tcc Ser	gca Ala	gcg Ala	ttt Phe 725	gtt Val	2515
gcc Ala	tcc Ser	act Thr	ctg Leu 730	atg Met	gag Glu	tat Tyr	ggt Gly	ggt Gly 735	gtt Val	cct Pro	cag Gln	tcc Ser	gca Ala 740	act Thr	ccg Pro	2563
gag Glu	tct Ser	ctt Leu 745	ctg Leu	aaa Lys	gaa Glu	gct Ala	atc Ile 750	cat His	gtt Val	ata Ile	agc Ser	tgt Cys 755	ggc Gly	tat Tyr	gag Glu	2611
gac Asp	aag Lys 760	act Thr	gaa Glu	tgg Trp	gga Gly	act Thr 765	gag Glu	atc Ile	Gly 999	tgg Trp	atc Ile 770	tac Tyr	ggt Gly	tct Ser	gtg Val	2659
aca Thr 775	gaa Glu	gac Asp	att Ile	ctc Leu	acc Thr 780	gga Gly	ttc Phe	aag Lys	atg Met	cac His 785	gcg Ala	cga Arg	ggc	tgg Trp	cgg Arg 790	2707
tcg Ser	atc Ile	tac Tyr	tgc Cys	atg Met 795	ccc Pro	aag Lys	cgg Arg	cca Pro	gct Ala 800	ttc Phe	aag Lys	ggg Gly	tct Ser	gcc Ala 805	ccc Pro	2755
atc Ile	aat Asn	ctt Leu	tcg Ser 810	gac Asp	cgt Arg	ctg Leu	aac Asn	cag Gln 815	gtg Val	ctc Leu	cgg Arg	tgg Trp	gct Ala 820	ctt Leu	gj aaa	2803
tcc Ser	gtg Val	gag Glu 825	atc Ile	ctc Leu	ttc Phe	agc Ser	cgg Arg 830	cac His	tgc Cys	ccc Pro	ctg Leu	tgg Trp 835	tac Tyr	ggc Gly	tac Tyr	2851
Gly	999 Gly 840	cgg Arg	ctc Leu	aag Lys	ttc Phe	ctg Leu 845	gag Glu	aga Arg	ttc Phe	gcg Ala	tac Tyr 850	atc Ile	aac Asn	acc Thr	acc Thr	2899
atc Ile 855	tac Tyr	ccg Pro	ctc Leu	acg Thr	tcc Ser 860	atc Ile	ccg Pro	ctt Leu	Leu	atc Ile 865	tac Tyr	tgc Cys	atc Ile	ctg Leu	ccc Pro 870	2947

gcc atc tgt ctg Ala Ile Cys Leu	ctc acc gga Leu Thr Gly 875	aag ttc atc Lys Phe Ile 880	att cca gag atc	agc aac 2995 Ser Asn 885
ttc gcc agc atc Phe Ala Ser Ile 890	tgg ttc atc Trp Phe Ile	tcc ctc ttc Ser Leu Phe 895	atc tcg atc ttc Ile Ser Ile Phe 900	Ala Thr
ggc atc ctg gag Gly Ile Leu Glu 905	atg agg tgg Met Arg Trp	agc ggg gtg Ser Gly Val 910	ggc atc gac gag Gly Ile Asp Glu 915	tgg tgg 3091 Trp Trp
agg aac gag cag Arg Asn Glu Gln 920	ttc tgg gtg Phe Trp Val 925	atc ggg ggc Ile Gly Gly	atc tcc gcg cac Ile Ser Ala His 930	ctc ttc 3139 Leu Phe
gcc gtg ttc cag Ala Val Phe Gln 935	ggc ctg ctc Gly Leu Leu 940	aag gtg ctg Lys Val Leu	gcc ggc atc gac Ala Gly Ile Asp 945	acc aac 3187 Thr Asn 950
ttc acc gtc acc Phe Thr Val Thr	tcc aag gcc Ser Lys Ala 955	tcg gac gag Ser Asp Glu 960	gac ggc gac ttc Asp Gly Asp Phe	gcg gag 3235 Ala Glu 965
ctg tac atg ttc Leu Tyr Met Phe : 970	aag tgg acg Lys Trp Thr	acg ctc ctg Thr Leu Leu 975	atc ccg ccc acc Ile Pro Pro Thr 980	acc atc 3283 Thr Ile
ctg atc atc aac Leu Ile Ile Asn : 985	ctg gtc ggc Leu Val Gly	gtc gtc gcc Val Val Ala 990	ggc atc tcc tac Gly Ile Ser Tyr 995	gcc atc 3331 Ala Ile
aac agc gga tac (Asn Ser Gly Tyr (1000	cag tcg tgg Gln Ser Trp 1005	Gly Pro Leu	ttc ggc aag ctc Phe Gly Lys Leu 1010	ttc ttc 3379 Phe Phe
gcc ttc tgg gtc a Ala Phe Trp Val 1 1015	atc gtc cac Ile Val His 1020	ctg tac ccg Leu Tyr Pro	ttc ctc aag ggc Phe Leu Lys Gly 1025	ctc atg 3427 Leu Met 1030
ggc agg cag aac of Gly Arg Gln Asn i	cge acc ccg Arg Thr Pro 1035	acc atc gtc Thr Ile Val 1040	Val Val Trp Ala	atc ctg 3475 Ile Leu 1045
ctg gcg tcc atc t Leu Ala Ser Ile I 1050	ttc tcc ttg Phe Ser Leu	ctg tgg gtt Leu Trp Val 1055	cgc atc gac ccc Arg Ile Asp Pro 106	Phe Thr
acc cgc gtc act of Thr Arg Val Thr (t 3566
gctagggaag tggaag gtctgttaag ttatat	tatat ataago	agca agtggcg	tta tttacagcta	gtacagacc 3686
agtggatatt gtttadaaaaaaaaaaaaaaaaaaaaaaa			ata tgcattcttt (tgttgatata 3746 3773

<210> 6 <211> 1077 <212> PRT <213> Zea mays

<400> 6 Met Glu Gly Asp Ala Asp Gly Val Lys Ser Gly Arg Arg Gly Gly Gly 10 Gln Val Cys Gln Ile Cys Gly Asp Gly Val Gly Thr Thr Ala Glu Gly 25 Asp Val Phe Ala Ala Cys Asp Val Cys Gly Phe Pro Val Cys Arg Pro 40 Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln Ala Cys Pro Gln Cys Lys Thr Lys Tyr Lys Arg His Lys Gly Ser Pro Ala Ile Arg Gly Glu 75 Glu Gly Asp Asp Thr Asp Ala Asp Ser Asp Phe Asn Tyr Leu Ala Ser 90 Gly Asn Glu Asp Gln Lys Gln Lys Ile Ala Asp Arg Met Arg Ser Trp 105 Arg Met Asn Val Gly Gly Ser Gly Asp Val Gly Arg Pro Lys Tyr Asp 120 Ser Gly Glu Ile Gly Leu Thr Lys Tyr Asp Ser Gly Glu Ile Pro Arg 135 Gly Tyr Ile Pro Ser Val Thr Asn Ser Gln Ile Ser Gly Glu Ile Pro 150 Gly Ala Ser Pro Asp His His Met Met Ser Pro Thr Gly Asn Ile Gly 165 170 Lys Arg Ala Pro Phe Pro Tyr Val Asn His Ser Pro Asn Pro Ser Arg 185 Glu Phe Ser Gly Ser Ile Gly Asn Val Ala Trp Lys Glu Arg Val Asp 200 205 Gly Trp Lys Met Lys Gln Asp Lys Gly Thr Ile Pro Met Thr Asn Gly 215 220 Thr Ser Ile Ala Pro Ser Glu Gly Arg Gly Val Gly Asp Ile Asp Ala 230 235 Ser Thr Asp Tyr Asn Met Glu Asp Ala Leu Leu Asn Asp Glu Thr Arg 250 Gln Pro Leu Ser Arg Lys Val Pro Leu Pro Ser Ser Arg Ile Asn Pro 265 Tyr Arg Met Val Ile Val Leu Arg Leu Ile Val Leu Ser Ile Phe Leu 280 285 His Tyr Arg Ile Thr Asn Pro Val Arg Asn Ala Tyr Pro Leu Trp Leu 295 300 Leu Ser Val Ile Cys Glu Ile Trp Phe Ala Leu Ser Trp Ile Leu Asp 310 315 Gln Phe Pro Lys Trp Phe Pro Ile Asn Arg Glu Thr Tyr Leu Asp Arg 325 330 Leu Ala Leu Arg Tyr Asp Arg Glu Gly Glu Pro Ser Gln Leu Ala Ala 345 Val Asp Ile Phe Val Ser Thr Val Asp Pro Met Lys Glu Pro Pro Leu 360 Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp Tyr Pro Val 375 Asp Lys Val Ser Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr 390 395 Phe Asp Ala Leu Ala Glu Thr Ser Glu Phe Ala Arg Lys Trp Val Pro 405 410

Phe	. Val	Lys	Lys 420		Asn	Ile	Glu	Pro		Ala	Pro	Glu	Trp		Phe
Ser	Gln	Lys 435	Ile	Asp	Tyr	Leu	Lys 440		Lys	Val	His	Pro	Ser	Phe	Val
Lys	Asp 450	Arg	Arg	Ala	Met	Lys 455		Glu	Tyr	Glu	Glu 460	Phe	Lys	Val	Arg
Val 465	Asn	Gly	Leu	Val	Ala 470	Lys	Ala	Gln	Lys	Val 475			Glu	Gly	Trp
			Asp	485					490					495	His
Pro	Gly	Met	Ile 500	Gln	Val	Phe	Leu	Gly 505		Ser	Gly	Gly	Leu 510	Asp	Thr
		515					520					525		_	_
	530		Gln			535					540				
545			Ala		550					555					560
			Tyr	565					570					575	
			Asp 580					585					590		
		595					600					605			_
-	610		Phe			615					620	_	_		
625			Tyr		630					635					640
			Glu	645					650					655	
			Gly 660					665					670		
		675	Lys				680					685			
	690		Asp			695					700				_
705			Leu		710					715					720
			Ala	725					730				_	735	
			Ala 740					745					750		
		755	Gly				760					765			
	770		Gly			775					780				
785			Gly		790					795					800
			Ser	805					810					815	
			Ala 820					825		•			830		_
		835	Tyr				840					845			
	850		Ile			855					860				
865	-1-	-76	***	u	870	n.a	116	cys	neu	875	IIIE	ату	nys	rue '	880 116

- 16 -

```
Ile Pro Glu Ile Ser Asn Phe Ala Ser Ile Trp Phe Ile Ser Leu Phe
                 885
                                     890
 Ile Ser Ile Phe Ala Thr Gly Ile Leu Glu Met Arg Trp Ser Gly Val
             900
                                 905
 Gly Ile Asp Glu Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly
                            920
 Ile Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu
                         935
 Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu
                     950
 Asp Gly Asp Phe Ala Glu Leu Tyr Met Phe Lys Trp Thr Thr Leu Leu
 Ile Pro Pro Thr Thr Ile Leu Ile Ile Asn Leu Val Gly Val Val Ala
                                985
 Gly Ile Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu
                             1000
 Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro
                       1015
 Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val
 1025
                     1030
                                        1035
 Val Val Trp Ala Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val
                1045
                                    1050
 Arg Ile Asp Pro Phe Thr Thr Arg Val Thr Gly Pro Asp Thr Gln Thr
            1060
                                1065
 Cys Gly Ile Asn Cys
        1075
       <210> 7
       <211> 25
       <212> DNA
       <213> Zea mays
      <400> 7
atggagggg acgcggacgg cgtga
25
      <210> 8
       <211> 25
       <212> DNA
      <213> Zea mays
      <400> 8
ctagcagttg atgccacacg tctgg
      <210> 9
      <211> 3780
      <212> DNA
      <213> Zea mays
      <220>
      <221> CDS
      <222> (201)...(3423)
      <400> 9
gtcgacccac gcgtccgcag cagcagaagc actgcgcggc attgcagcga tcgagcggga
                                                                      60
ggaatttggg gcatggtggt cgccaacgcc gctcggatct agaggcccgc acgggccgat
                                                                     120
tggtctccgc ccgcctcgtc ggtgttggtg tcgttggcgt gtggagccgt ctcggtggga
```

- 17 -

gca	agcg	gga	ggga	agcg	gag a	atg g Met 1	ncg g	gcc a Ala	ac a Asn	ag g Lys 5	ggg a	atg q Met	gtg g Val	gcg (Ala	ggc tc Gly s 10	g er	233
cac His	c aad s Asr	e ego n Aro	e aad Asi 15	I GI	tto Phe	gto Val	atg Met	ato Ile	Arg	cac His	gaç S Asp	ggc Gl	gat Asp	Val	g ccg L Pro		281
Gl ⁷	teg Ser	gct Ala 30	т губ	g cco	aca Thr	aag Lys	agt Ser 35	Ala	aat Asn	gga Gly	cag Gln	gto Val	Cys	cag Glr	att Ile		329
tgo Cys	ggt Gly 45	ASE	tct Ser	gtg Val	ggt Gly	gtt Val 50	Ser	gcc Ala	act Thr	ggt	gat Asp 55	Val	ttt Phe	gtt Val	gcc Ala		377
tgc Cys 60	ASI	gag Glu	tgt Cys	gee Ala	Phe 65	Pro	gtc Val	tgc Cys	cgc Arg	cca Pro 70	Cys	tat Tyr	gag Glu	tat Tyr	gag Glu 75		425
cgc Arg	aag Lys	gag Glu	Gly ggg	aac Asn 80	caa Gln	tgc Cys	tgc Cys	ccc Pro	cag Gln 85	tgc Cys	aag Lys	act Thr	aga Arg	tac Tyr 90	aag Lys		473
aga Arg	cag Gln	aaa Lys	ggt Gly 95	ser	cct Pro	cga Arg	gtt Val	cat His 100	ggt Gly	gat Asp	gag Glu	gat Asp	gag Glu 105	gaa Glu	gat Asp		521
gtt Val	gat Asp	gac Asp 110	cta Leu	gac Asp	aat Asn	gaa Glu	ttc Phe 115	aac Asn	tac Tyr	aag Lys	caa Gln	ggc Gly 120	agt Ser	ggg ggg	aaa Lys		569
ggc Gly	cca Pro 125	gag Glu	tgg Trp	caa Gln	ctg Leu	caa Gln 130	gga Gly	gat Asp	gat Asp	gct Ala	gat Asp 135	ctg Leu	tct Ser	tca Ser	tct Ser		617
gct Ala 140	cgc Arg	cat His	gag Glu	cca Pro	cat His 145	cat His	cgg Arg	att Ile	cca Pro	cgc Arg 150	ctg Leu	aca Thr	agc Ser	ggt Gly	caa Gln 155		665
cag Gln	ata Ile	tct Ser	gga Gly	gag Glu 160	att Ile	cct Pro	gat Asp	gct Ala	tcc Ser 165	cct Pro	gac Asp	cgt Arg	cat His	tct Ser 170	atc Ile		713
cgc Arg	agt Ser	cca Pro	aca Thr 175	tcg Ser	agc Ser	tat Tyr	gtt Val	gat Asp 180	cca Pro	agc Ser	gtc Val	cca Pro	gtt Val 185	cct Pro	gtg Val		761
agg Arg	att Ile	gtg Val 190	gac Asp	ccc Pro	tcg Ser	Lys	gac Asp 195	ttg Leu	aat Asn	tcc Ser	tat Tyr	999 Gly 200	ctt Leu	aat Asn	agt Ser		809
gtt Val	gac Asp 205	tgg Trp	aag Lys	gaa Glu	aga Arg	gtt Val 210	gag Glu	agc Ser	tgg Tṛp	agg Arg	gtt Val 215	aaa Lys	cag Gln	gac Asp	aaa Lys	;	857
aat Asn	atg Met	atg Met	caa Gln	gtg Val	act Thr	aat Asn	aaa Lys	tat Tyr	cca Pro	gag Glu	gct Ala	aga Arg	gga Gly	gga Gly	gac Asp	!	905



- 18 -

220	ı				225					230)				235	
atg Met	gag Glu	ggg Gly	act Thr	ggc Gly 240	Ser	aat Asn	gga Gly	gaa Glu	nat Xaa 245	Met	caa Gln	atg Met	gtt Val	gat Asp 250	Asp	953
gca Ala	. cgg . Arg	cta Lev	cct Pro 255	Leu	agc Ser	cgt Arg	atc Ile	gtg Val 260	cca Pro	att Ile	tcc Ser	tca Ser	aac Asn 265	Gln	ctc Leu	1001
aac Asn	ctt Leu	tac Tyr 270	Arg	gta Val	gtg Val	atc Ile	att Ile 275	Leu	cgt Arg	ctt Leu	atc Ile	atc Ile 280	Leu	tgc Cys	ttc Phe	1049
ttc Phe	ttc Phe 285	cag Gln	tat Tyr	cgt Arg	gtc Val	agt Ser 290	cat His	cca Pro	gtg Val	cgt Arg	gat Asp 295	gct Ala	tat Tyr	gga Gly	tta Leu	1097
tgg Trp 300	Leu	gta Val	tct Ser	gtt Val	atc Ile 305	tgc Cys	gag Glu	gtc Val	tgg Trp	ttt Phe 310	gcc Ala	ttg Leu	tct Ser	tgg Trp	ctt Leu 315	1145
cta Leu	gat Asp	cag Gln	ttc Phe	cca Pro 320	aaa Lys	tgg Trp	tat Tyr	cca Pro	atc Ile 325	aac Asn	cgt Arg	gag Glu	aca Thr	tat Tyr 330	ctt Leu	1193
gac Asp	agg Arg	ctt Leu	gca Ala 335	ttg Leu	agg Arg	tat Tyr	gat Asp	aga Arg 340	gag Glu	gga Gly	gag Glu	cca Pro	tca Ser 345	cag Gln	ctg Leu	1241
gct Ala	ccc Pro	att Ile 350	gat Asp	gtc Val	ttc Phe	gtc Val	agt Ser 355	aca Thr	gtg Val	gat Asp	cca Pro	ttg Leu 360	aag Lys	gaa Glu	cct Pro	1289
cca Pro	ctg Leu 365	atc Ile	aca Thr	gcc Ala	aac Asn	act Thr 370	gtt Val	ttg Leu	tcc Ser	att Ile	ctt Leu 375	tct Ser	gtg Val	gat Asp	tac Tyr	1337
cct Pro 380	gtt Val	gac Asp	aaa Lys	gtg Val	tca Ser 385	tgc Cys	tat Tyr	gtt Val	tct Ser	gat Asp 390	gat Asp	ggt Gly	tca Ser	gct Ala	atg Met 395	1385
ctg Leu	act Thr	ttt Phe	gag Glu	tct Ser 400	ctc Leu	tca Ser	gaa Glu	acc Thr	gca Ala 405	gaa Glu	ttt Phe	gct Ala	aga Arg	aag Lys 410	tgg Trp	1433
gtt Val	ccc Pro	ttt Phe	tgt Cys 415	aag Lys	aag Lys	cac His	aat Asn	att Ile 420	gaa Glu	cca Pro	aga Arg	gct Ala	cca Pro 425	gaa Glu	ttt Phe	1481
tac Tyr	ttt Phe	gct Ala 430	caa Gln	aaa Lys	ata Ile	gat Asp	tac Tyr 435	ctg Leu	aag Lys	gac Asp	aaa Lys	att Ile 440	caa Gln	cct Pro	tca Ser	1529
Phe	gtt Val 445	aag Lys	gaa Glu	aga Arg	cgc Arg	gca Ala 450	atg Met	aag Lys	agg Arg	gag Glu	tat Tyr 455	gaa Glu	gaa Glu	ttc Phe	aaa Lys	1577

- 19 -

gta Val 460	Arg	atc Ile	aat Asn	gcc	Leu 465	Val	gcc Ala	aaa Lys	gca Ala	cag Gln 470	Lys	gtg Val	cct Pro	gaa Glu	gag Glu 475	1625
gly aaa	tgg Trp	acc Thr	atg Met	gct Ala 480	gat Asp	gga Gly	act Thr	gca Ala	tgg Trp 485	cct Pro	Gly 999	aat Asn	aat Asn	cct Pro 490	Arg	1673
gac Asp	cat	cct Pro	ggc Gly 495	atg Met	att Ile	cag Gln	gtt Val	ttc Phe 500	ttg Leu	gly aaa	cac His	agt Ser	ggt Gly 505	gly	ctc Leu	1721
gac Asp	act Thr	gat Asp 510	gga Gly	aat Asn	gag Glu	tta Leu	cca Pro 515	cgt Arg	ctt Leu	gtc Val	tat Tyr	gtc Val 520	tct Ser	cgt Arg	gaa Glu	1769
aag Lys	aga Arg 525	cca Pro	ggc Gly	ttt Phe	cag Gln	cat His 530	cac His	aag Lys	aag Lys	gct Ala	ggt Gly 535	gca Ala	atg Met	aat Asn	gcg Ala	1817
ctg Leu 540	att Ile	cgt Arg	gta Val	tct Ser	gct Ala 545	gtg Val	ctg Leu	aca Thr	aat Asn	ggt Gly 550	gcc Ala	tat Tyr	ctt Leu	ctc Leu	aat Asn 555	1865
gtg Val	gat Asp	tgc Cys	gac Asp	cat His 560	tac Tyr	ttc Phe	aat Asn	agc Ser	agc Ser 565	aaa Lys	gct Ala	ctt Leu	aga Arg	gaa Glu 570	gca Ala	1913
atg Met	tgc Cys	ttc Phe	atg Met 575	atg Met	gat Asp	ccg Pro	gct Ala	cta Leu 580	gga Gly	agg Arg	aaa Lys	act Thr	tgt Cys 585	tat Tyr	gta Val	1961
caa Gln	ttt Phe	cca Pro 590	cag Gln	aga Arg	ttt Phe	gat Asp	ggc Gly 595	att Ile	gac Asp	ttg Leu	cac His	gat Asp 600	cga Arg	tat Tyr	gct Ala	2009
aat Asn	cgg Arg 605	aac Asn	ata Ile	gtt Val	ttc Phe	ttt Phe 610	gat Asp	atc Ile	aac Asn	atg Met	aaa Lys 615	ggt Gly	ctg Leu	gat Asp	ggc Gly	2057
att Ile 620	cag Gln	ggt Gly	cca Pro	gtt Val	tac Tyr 625	gtg Val	gga Gly	aca Thr	gga Gly	tgc Cys 630	tgt Cys	ttc Phe	aat Asn	aga Arg	cag Gln 635	2105
gct Ala	ttg Leu	tat Tyr	gga Gly	tac Tyr 640	gat Asp	cct Pro	gtt Val	ttg Leu	act Thr 645	gaa Glu	gct Ala	gat Asp	ctg Leu	gag Glu 650	cca Pro	2153
aac Asn	att Ile	gtt Val	att Ile 655	aag Lys	agc Ser	tgc Cys	tgt Cys	ggt Gly 660	aga Arg	agg Arg	aag Lys	aaa Lys	aag Lys 665	aac Asn	aag Lys	2201
agt Ser	tat Tyr	atg Met 670	gat Asp	agt Ser	caa Gln	agc Ser	cgt Arg 675	att Ile	atg Met	aag Lys	aga Arg	aca Thr 680	gaa Glu	tct Ser	tca Ser	2249
gct Ala	ccc Pro	atc Ile	ttc Phe	aat Asn	atg Met	gaa Glu	gac Asp	atc Ile	gaa Glu	gag Glu	ggt Gly	att Ile	gaa Glu	ggt Gly	tac Tyr	2297

- 20 *-*

	685					690					695	;				
gag Glu 700	gat Asp	gaa Glu	agg Arg	tca Ser	gtg Val 705	Leu	atg Met	tcc Ser	cag Gln	agg Arg 710	Lys	ttg Leu	gag Glu	aaa Lys	cgc Arg 715	2345
Phe	Gly	Gln	Ser	Pro 720	Ile	Phe	Ile	Ala	Ser 725	Thr	Phe		Thr	Gln 730	Gly	2393
ggc	ata Ile	cca Pro	cct Pro 735	Ser	aca Thr	aac Asn	cca Pro	gct Ala 740	tct Ser	cta Leu	cta Leu	aag Lys	gaa Glu 745	gct Ala	atc Ile	2441
cat His	gtc Val	atc Ile 750	agt Ser	tgt Cys	gga Gly	tat Tyr	gag Glu 755	gac Asp	aaa Lys	act Thr	gaa Glu	tgg Trp 760	gga Gly	aaa Lys	gag Glu	2489
att Ile	ggc Gly 765	tgg Trp	atc Ile	tat Tyr	ggt Gly	tca Ser 770	gta Val	acg Thr	gag Glu	gat Asp	att Ile 775	ctg Leu	act Thr	Gly 999	ttt Phe	2537
aaa Lys 780	atg Met	cat His	gca Ala	agg Arg	ggc Gly 785	tgg Trp	caa Gln	tca Ser	atc Ile	tac Tyr 790	tgc Cys	atg Met	cca Pro	cca Pro	cga Arg 795	2585
ect Pro	tgt Cys	ttc Phe	aag Lys	ggt Gly 800	tct Ser	gca Ala	cca Pro	atc Ile	aat Asn 805	ctt Leu	tcc Ser	gat Asp	cgt Arg	ctt Leu 810	aat Asn	2633
cag Gln	gtg Val	ctc Leu	cgt Arg 815	tgg Trp	gct Ala	ctt Leu	gl ^à aaa	tca Ser 820	gtg Val	gaa Glu	att Ile	ctg Leu	ctt Leu 825	agt Ser	aga Arg	2681
cat His	tgt Cys	cct Pro 830	atc Ile	tgg Trp	tat Tyr	ggt Gly	tac Tyr 835	aat Asn	gga Gly	cga Arg	ttg Leu	aag Lys 840	ctt Leu	ttg Leu	gag Glu	2729
agg Arg	ctg Leu 845	gct Ala	tac Tyr	atc Ile	aac Asn	act Thr 850	att Ile	gta Val	tat Tyr	cca Pro	atc Ile 855	aca Thr	tcc Ser	att Ile	ccg Pro	2777
ctt Leu 860	att Ile	gcc Ala	tat Tyr	tgt Cys	gtg Val 865	ctt Leu	ccc Pro	gct Ala	atc Ile	tgc Cys 870	ctc Leu	ctt Leu	acc Thr	aat Asn	aaa Lys 875	2825
ttt . Phe	atc Ile	att Ile	cct Pro	gag Glu 880	att Ile	agc Ser	aat Asn	tat Tyr	gct Ala 885	gly aga	atg Met	ttc Phe	ttc Phe	att Ile 890	ctt Leu	2873
ctt Leu	ttc Phe	Ala	tcc Ser 895	att Ile	ttt Phe	gcc Ala	act Thr	ggt Gly 900	ata Ile	ttg Leu	gag Glu	Leu	aga Arg 905	tgg Trp	agt Ser	2921
ggt g Gly v	gtt Val	ggc Gly 910	att Ile	gaa Glu	gat Asp	tgg Trp	tgg Trp 915	aga Arg	aat Asn	gag Glu	cag Gln	ttt Phe 920	tgg Trp	gtt Val	att Ile	2969

- 21 -

- 21 -	
ggt ggc acc tct gcc cat ctc ttc gca gtg ttc cag ggt ctg ctg aaa Gly Gly Thr Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys 925 930 935	3017
gtg ttg gct ggg att gat acc aac ttc aca gtt acc tca aag gca tct Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser 940 945 950 955	3065
gat gag gat ggc gac ttt gct gag cta tat gtg ttc aag tgg acc agt Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser 960 965 970	3113
ttg ctc att cct ccg acc act gtt ctt gtc att aac ctg gtc gga atg Leu Leu Ile Pro Pro Thr Thr Val Leu Val Ile Asn Leu Val Gly Met 975 980 985	3161
gtg gca gga att tct tat gcc att aac agt ggc tac caa tcc tgg ggt Val Ala Gly Ile Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly 990 995 1000	3209
ccg ctc ttt gga aag ctg ttc ttc tcg atc tgg gtg atc ctc cat ctc Pro Leu Phe Gly Lys Leu Phe Phe Ser Ile Trp Val Ile Leu His Leu 1005 1010 1015	3257
tac ccc ttc ctc aag ggt ctc atg gga agg cag aac cgc aca cca aca Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr 1020 1025 1030 1035	3305
atc gtc att gtc tgg tcc atc ctt ctt gca tct atc ttc tcc ttg ctg Ile Val Ile Val Trp Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu 1040 1045 1050	3353
tgg gtg aag atc gat cct ttc atc tcc ccg aca cag aaa gct gct gcc Trp Val Lys Ile Asp Pro Phe Ile Ser Pro Thr Gln Lys Ala Ala 1055 1060 1065	3401
ttg ggg caa tgt ggc gtc aac t gctgatcgag acagtgactc ttatttgaag Leu Gly Gln Cys Gly Val Asn 1070	3453
aggeteaate aagatetgee eeetegtgta aatacetgag gaggetagat gggaatteet titgtigtag gtgaggatgg attigeatet aagttatgee tetgtieatt agettettee gtgeeggtge tgetgeggae taagaateae ggageettte taeetteeat gtagegeeag eeageagegt aagatgtgaa tittgaagti tigitatgeg tgeagtitat tgitttagag taaattatea titgittigtg ggaaetgite acaegageit ataatggeaa tgetgitati taaaaaaaaaa aaaaaaaggg eggeege	3513 3573 3633 3693 3753 3780
<210> 10 <211> 1075 <212> PRT <213> Zea mays	
<pre></pre>	
Phe Val Met Ile Arg His Asp Gly Asp Val Pro Gly Ser Ala Lys Pro 20 25 30	•

Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Ser Val

		35					40					45			
	50		Ala			55					60				
65			Cys		70					75				_	80
			Pro	85					90					95	
Pro	Arg	Val	His 100	Gly	Asp	Glu	Asp	Glu 105		Asp	Val	Asp	Asp	Leu	Asp
Asn	Glu	Phe 115	Asn	Tyr	Lys	Gln	Gly 120		Gly	Lys	Gly	Pro	Glu	Trp	Gln
Leu	Gln 130	Gly	Asp	Asp	Ala	Asp 135		Ser	Ser	Ser	Ala	Arg		Glu	Pro
His 145	His	Arg	Ile	Pro	Arg 150		Thr	Ser	Gly	Gln 155			Ser	Gly	Glu 160
Ile	Pro	Asp	Ala	Ser 165		Asp	Arg	His	Ser	Ile	Arg	Ser	Pro	Thr 175	Ser
Ser	Tyr	Val	Asp 180	Pro	Ser	Val	Pro	Val 185	Pro		Arg	Ile	Val 190	Asp	Pro
Ser	Lys	Asp 195	Leu	Asn	Ser	Tyr	Gly 200			Ser	Val	Asp 205	Trp	Lys	Glu
Arg	Val 210	Glu	Ser	Trp	Arg	Val 215	Lys	Gln	Asp	Lys	Asn 220		Met	Gln	Val
Thr 225	Asn	Lys	Tyr	Pro	Glu 230	Ala	Arg	Gly	Gly	Asp 235	Met	Glu	Gly	Thr	Gly 240
Ser	Asn	Gly	Glu	Xaa 245	Met	Gln	Met	Val	Asp 250		Ala	Arg	Leu	Pro 255	Leu
Ser	Arg	Ile	Val 260	Pro	Ile	Ser	Ser	Asn 265		Leu	Asn	Leu	Tyr 270	Arg	Val
Val	Ile	Ile 275	Leu	Arg	Leu	Ile	Ile 280	Leu	Cys	Phe	Phe	Phe 285		Tyr	Arg
Val	Ser 290	His	Pro	Val	Arg	Asp 295	Ala	Tyr	Gly	Leu	Trp	Leu	Val	Ser	Val
305			Val		310					315					320
			Pro	325					330					335	Leu
Arg	Tyr	qaA	Arg 340	Glu	Gly	Glu	Pro	Ser 345	Gln	Leu	Ala	Pro	Ile 350	Asp	Val
Phe	Val	Ser 355	Thr	Val	Asp	Pro	Leu 360	Lys	Glu	Pro	Pro	Leu 365	Ile	Thr	Ala
Asn	Thr 370	Val	Leu	Ser	Ile	Leu 375	Ser	Val	Asp	Tyr	Pro 380	Val	Asp	Lys	Val
Ser 385	Cys	Tyr	Val	Ser	Asp 390	Asp	Gly	Ser	Ala	Met 395	Leu	Thr	Phe	Glu	Ser 400
Leu	Ser	Glu	Thr	Ala 405	Glu	Phe	Ala	Arg	Lys 410	Trp	Val	Pro	Phe	Cys 415	Lys
Lys	His	Asn	Ile 420	Glu	Pro	Arg	Ala	Pro 425	Glu	Phe	Tyr	Phe	Ala 430	Gln	Lys
Ile		Tyr 435	Leu	Lys	Asp	Lys	Ile 440	Gln	Pro	Ser	Phe	Val 445		Glu	Arg
	Ala 450	Met	Lys	Arg	Glu	Tyr 455	Glu	Glu	Phe	Lys	Val 460	Arg	Ile	Asn	Ala
Leu 465	Val	Ala	Lys	Ala	Gln 470	Lys	Val	Pro	Glu	Glu 475		Trp	Thr	Met	Ala 480
Asp	Gly	Thr	Ala	Trp 485	Pro	Gly	Asn	Asn	Pro 490		Asp	His	Pro	Gly 495	
Ile	Gln	Val	Phe	Leu	Gly	His	Ser	Gly	Gly	Leu	Asp	Thr	Asp		Asn

			500)				505	:				E 1 0		
Gli	ı Leı) Pro	Arg		Val	Туг	Val	. Ser		g Glu	Lys	Arg		Gly	Phe
Glr	His 530	His	Lys	Lys	Ala	Gly 535	Ala		Asn	Ala	Leu 540	Ile	Arg	Val	Ser
545	•				550					555	Val	Asp			His 560
			Ser	565					570					575	Met
			580					585					590	Gln	Arg
		595					600	1				605	•		
	610	•	Ile			615					620				
625			Thr		630					635					640
			Leu	645					650					655	_
			Gly 660					665					670	_	
		675					680					685			
	690		Ile			695					700				
705			Ser		710					715					720
			Ala	725					730					735	Ser
			Ala 740					745					750		_
		755	Asp				760					765			
	770		Thr			775					780				-
785			Ser		790					795					800
			Ile	805					810					815	
			Ser 820					825					830		_
		835	Asn				840					845		_	
	850		Val			855					860				
Val 865	Leu	Pro	Ala	Ile	Cys 870	Leu	Leu	Thr	Asn	Lys 875	Phe	Ile	Ile	Pro	
	Ser	Asn	Tyr	Ala 885		Met	Phe	Phe	Ile 890		Leu	Phe	Ala	Ser 895	880 Ile
Phe	Ala	Thr	Gly 900	Ile	Leu	Glu	Leu	Arg 905		Ser	Gly	Val	Gly 910		Glu
Asp	Trp	Trp 915	Arg	Asn	Glu	Gln	Phe 920		Val	Ile	Gly	Gly 925	Thr	Ser	Ala
	930		Ala			935					940	Leu			
945			Phe		950					955	qaA				960
Phe	Ala	Glu	Leu	Tyr	Val	Phe	Lys	Trp	Thr	Ser	Leu	Leu	Ile	Pro	Pro

- 24 -

```
970
                965
Thr Thr Val Leu Val Ile Asn Leu Val Gly Met Val Ala Gly Ile Ser
                     985
           980
                                                   990
Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys
                           1000
                                              1005
Leu Phe Phe Ser Ile Trp Val Ile Leu His Leu Tyr Pro Phe Leu Lys
                        1015
                                          1020
Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp
                    1030
                                       1035
Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys Ile Asp
                1045
                                   1050
Pro Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala Leu Gly Gln Cys Gly
            1060
                               1065
Val Asn Cys
        1075
      <210> 11
      <211> 25
      <212> DNA
      <213> Zea mays
      <400> 11
atggcggcca acaaggggat ggtgg
25
      <210> 12
      <211> 25
      <212> DNA
      <213> Zea mays
      <400> 12
tcagcagttg acgccacatt gcccc
25
      <210> 13
      <211> 3725
      <212> DNA
      <213> Zea mays
      <220>
      <221> CDS
      <222> (179)...(3398)
gcagcagcag caccaccact gcgcggcatt gcagcgagca agcgggaggg atctggggca
tggtggggt cgctgccgct gccgctcgga tctagagggc cgcacgggct gattgccctc
                                                                    120
                                                                    178
cgccggcctc gtcggtgtcg gtggagtgtg aatcggtgtg tgtaggagga gcgcggag
atg gcg gcc aac aag ggg atg gtg gca ggc tct cac aac cgc aac gag
                                                                    226
Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu
tto gto atg atc ogo cac gac ggo gac gog cot gto cog got aag coc
                                                                    274
Phe Val Met Ile Arg His Asp Gly Asp Ala Pro Val Pro Ala Lys Pro
             20
                                                                    322
 acg aag agt gcg aat ggg cag gtc tgc cag att tgt ggc gac act gtt
 Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Thr Val
                                                 45
                             40
```

WO 00/09706

ggc	gtt Val 50	tca Ser	gcc Ala	act Thr	ggt Gly	gat Asp 55	gtc Val	ttt Phe	gtt Val	gcc Ala	tgc Cys 60	aat Asn	gag Glu	tgt Cys	gcc Ala	370
ttc Phe 65	Pro	gtc Val	tgc Cys	cgc Arg	cct Pro 70	tgc Cys	tat Tyr	gag Glu	tac Tyr	gag Glu 75	cgc Arg	aag Lys	gaa Glu	ely aaa	aac Asn 80	418
Gln	Cys	Cys	cct Pro	Gln 85	Cys	Lys	Thr	Arg	Tyr 90	Lys	Arg	Gln	ГÀЗ	Gly 95	Ser	466
Pro	Arg	Val	cat His 100	Gly	Asp	Asp	Glu	Glu 105	Glu	Asp	Val	qaA	Asp 110	Leu	Asp	514
aat Asn	gaa Glu	ttc Phe 115	aac Asn	tat Tyr	aag Lys	caa Gln	ggc Gly 120	aat Asn	gly aaa	aag Lys	ggc	cca Pro 125	gag Glu	tgg Trp	cag Gln	562
Leu	Gln 130	Gly	gat Asp	Asp	Ala	Asp 135	Leu	Ser	Ser	Ser	Ala 140	Arg	His	Asp	Pro	610
His 145	His	Arg	att Ile	Pro	Arg 150	Leu	Thr	Ser	Gly	Gln 155	Gln	Ile	Ser	Gly	Glu 160	658
atc Ile	cct Pro	gat Asp	gca Ala	tcc Ser 165	cct Pro	gac Asp	cgt Arg	cat His	tct Ser 170	atc Ile	cgc Arg	agt Ser	cca Pro	aca Thr 175	tcg Ser	706
Ser	Tyr	Val	gat Asp 180	Pro	Ser	Val	Pro	Val 185	Pro	Val	Arg	Ile	Val 190	Asp	Pro	754
tcg Ser	aag Lys	gac Asp 195	ttg Leu	aat Asn	tcc Ser	tat Tyr	999 Gly 200	ctt Leu	aat Asn	agt Ser	gtt Val	gac Asp 205	tgg Trp	aag Lys	gaa Glu	802
			agc Ser													850
act Thr 225	aat Asn	aaa Lys	tat Tyr	cca Pro	gag Glu 230	gct Ala	aga Arg	gga Gly	gac Asp	atg Met 235	gag Glu	gly aaa	act Thr	ggc Gly	tca Ser 240	898
aat Asn	gga Gly	gaa Glu	gat Asp	atg Met 245	caa Gln	atg Met	gtt Val	gat Asp	gat Asp 250	gca Ala	cgc Arg	cta Leu	cct Pro	ttg Leu 255	agc Ser	946
cgc Arg	att Ile	gtg Val	cca Pro 260	att Ile	tcc Ser	tca Ser	aac Asn	cag Gln 265	ctc Leu	aac Asn	ctt Leu	tac Tyr	cgg Arg 270	ata Ile	gta Val	994
atc	att	ctc	cgt	ctt	atc	atc	ctg	tgc	ttc	ttc	ttc	caa	tat	cgt	atc	1042

- 26 -

								_	•							
Ile	Ile	Leu 275	Arg	Leu	Ile	Ile	Leu 280	Cys	Phe	Phe	Phe	Gln 285	Tyr	Arg	Ile	
agt Ser	cat His 290	cca Pro	gtg Val	cgt Arg	aat Asn	gct Ala 295	tat Tyr	gga Gly	ttg Leu	tgg Trp	cta Leu 300	gta Val	tct Ser	gtt Val	atc Ile	1090
tgt Cys 305	gag Glu	gtc Val	tgg Trp	ttt Phe	gcc Ala 310	ttg Leu	tcc Ser	tgg Trp	ctt Leu	cta Leu 315	gat Asp	cag Gln	ttc Phe	cca Pro	aaa Lys 320	1138
tgg Trp	tat Tyr	cca Pro	atc Ile	aac Asn 325	cgt Arg	gag Glu	aca Thr	tat Tyr	ctc Leu 330	gac Asp	agg Arg	ctt Leu	gca Ala	ttg Leu 335	agg Arg	1186
tat Tyr	gat Asp	aga Arg	gag Glu 340	gga Gly	gag Glu	cca Pro	tca Ser	cag Gln 345	ctg Leu	gct Ala	ccc Pro	att Ile	gat Asp 350	gtc Val	ttt Phe	1234
gtc Val	agt Ser	aca Thr 355	gtg Val	gat Asp	cca Pro	ttg Leu	aag Lys 360	gaa Glu	cct Pro	cca Pro	ctg Leu	atc Ile 365	aca Thr	gcc Ala	aac Asn	1282
act Thr	gtt Val 370	ttg Leu	tcc Ser	att Ile	ctt Leu	gct Ala 375	gtg Val	gat Asp	tac Tyr	cct Pro	gtt Val 380	gac Asp	aaa Lys	gtg Val	tca Ser	1330
tgc Cys 385	tat Tyr	gtt Val	tct Ser	gat Asp	gat Asp 390	ggc Gly	tca Ser	gct Ala	atg Met	ctg Leu 395	act Thr	ttt Phe	gag Glu	tct Ser	ctc Leu 400	1378
								aag Lys								1426
cac His	aat Asn	att Ile	gaa Glu 420	cca Pro	aga Arg	gct Ala	cca Pro	gaa Glu 425	ttt Phe	tac Tyr	ttt Phe	gct Ala	caa Gln 430	aaa Lys	ata Ile	1474
								cct Pro								1522
								ttc Phe								1570
								gaa Glu								1618
								cct Pro		_				_		1666
								202 GJA GGG		_		-			_	1714

WO 00/09706

	cca Pro															1762
	cac His 530															1810
	ctg Leu				-						_	_	_			1856
	: aat : Asn	-	-		-			_	-	_	_		_	_	_	1906
	gct Ala						-							_		1954
_	ggc Gly		_	-		_	_		-					_		2002
	gat Asp 610															2050
	gga Gly			_					-	_	_				_	2098
	gtt Val															2146
_	tgt Cys		_		-	-	_		_	_		_	_	_		2194
	cgt Arg		-	-	_		-			-					_	2242
_	gac Asp 690						_				_	_				2290
	atg Met															2338
	att lle	_				_										2386
aad	cca	gct	tct	cta	ctg	aag	gaa	gct	atc	cat	gtt	atc	agc	tgt	aaa	2434

- 28 -

Asn	Pro	Ala	Ser 740	Leu	Leu	Lys	Glu	Ala 745	Ile	His	Val	Ile	Ser 750	Сув	Gly	
														tat Tyr		2482
														aga Arg		2530
														ggt Gly		2578
														tgg Trp 815		2626
														tgg Trp		2674
														att Ile		2722
								_	_			_		tgt Cys		2770
														gag Glu		2818
														att Ile 895		2866
														gaa Glu		2914
														gcc Ala		2962
														att Ile		3010
														gac Asp		3058
_						_			_	_				ccg Pro 975		3106

act gtt ctt Thr Val Leu	gtc att Val Ile 980	aac ctg	g gtc gga 1 Val Gly 985	Met Val	gca gga Ala Gly	att tcg Ile Ser 990	g tat 3	3154
gcc att aac Ala Ile Asn 99	Ser Gly	tac caa Tyr Gln	tcc tgg Ser Trp 1000	ggt ccg	ctc ttt Leu Phe 100	Gly Lys	g ctg 3 s Leu	202
ttc ttc tcg Phe Phe Ser 1010	atc tgg Ile Trp	gtg ato Val Ile 101	Leu His	ctc tac Leu Tyr	Pro Phe	ctc aag Leu Lys	ggt 3 Gly	250
ctc atg ggc Leu Met Gly 1025	agg cag Arg Gln	aac cgc Asn Arg 1030	acg cca	aca atc Thr Ile 103	Val Ile	gtt tgg Val Trp	tcc 3 Ser 1040	298
atc ctc ctt Ile Leu Leu	gcg tct Ala Ser 104	Ile Phe	tcc ttg Ser Leu	ctg tgg Leu Trp 1050	gtg aag Val Lys	atc gat Ile Asp 105	Pro	346
ttc atc tcc Phe Ile Ser	ccg aca Pro Thr 1060	cag aaa Gln Lys	gct gcc Ala Ala 106	Ala Leu	ggg caa Gly Gln	tgt ggt Cys Gly 1070	gtg 3. Val	394
aac t gctga	ccag at	tgtgactc	ttatctg	aag aggc	tcagcc a	aagatctg	·c 3	448
cccctcgtgt a tctgcatcca a atcatggagc c atttttta a attcacacga a	agttatge etttetae :aegtggt	ct ctgtt ct tgctt gt ttatt	tatta gc gtagt gc gtttt ag	ttcttcgg tggccagc agtaaatt	tgccggt;	gct gctg att gtga	cagaca 3! attctg 36 gtaact 36	508 568 628 688 725
<210> <211>								
<212>		æ						
	_	5						
<400> Met Ala Ala 1		Gly Met	Val Ala	Gly Ser	His Asn	Arg Asn	Glu	
Phe Val Met	Ile Arg 20	His Asp	Gly Asp 25	Ala Pro	Val Pro	Ala Lys 30	Pro	
Thr Lys Ser 35	Ala Asn	Gly Gln	Val Cys 40	Gln Ile	Cys Gly	Asp Thr	Val	
Gly Val Ser 50	Ala Thr	Gly Asp 55	Val Phe	Val Ala	Cys Asn	Glu Cys	Ala	
Phe Pro Val 65	Cys Arg	Pro Cys	Tyr Glu	Tyr Glu 75	Arg Lys	Glu Gly	Asn 80	
Gln Cys Cys	Pro Gln 85	Cys Lys	Thr Arg	Tyr Lys	Arg Gln	Lys Gly	Ser	
Pro Arg Val	His Gly 100	Asp Asp	Glu Glu 105	Glu Asp	Val Asp		Asp	
Asn Glu Phe		Lys Gln		Gly Lys	Gly Pro		Gln	
Leu Gln Gly								

	130					135					140				
His 145	His	Arg	Ile	Pro	Arg 150	Leu	Thr	Ser	Gly	Gln 155	Gln	Ile	Ser	Gly	Glu 160
Ile	Pro	Asp	Ala	Ser 165	Pro	Asp	Arg	His	Ser 170	Ile	Arg	Ser	Pro	Thr 175	
Ser	Tyr	Val	Asp 180	Pro	Ser	Val	Pro	Val 185		Val	Arg	Ile	Val		Pro
Ser	Lys	Asp		Asn	Ser	Tyr	Gly 200		Asn	Ser	Val	Asp 205		Lys	Glu
Arg	Val 210		Ser	Trp	Arg	Val 215		Gln	Asp	Lys			Leu	Gln	Val
Thr 225	Asn	Lys	Tyr	Pro			Arg	Gly	Asp		220 Glu	Gly	Thr	Gly	
	Gly	Glu	Asp		230 Gln	Met	Val	Asp		235 Ala	Arg	Leu	Pro		240 Ser
Arg	Ile	Val		245 Ile	Ser	Ser	Asn		250 Leu	Asn	Leu	Tyr	_	255 Ile	Val
Ile	Ile		260 Arg	Leu	Ile	Ile		265 Cys	Phe	Phe	Phe		270 Tyr	Arg	Ile
Ser	His	275 Pro	Val	Arg	Asn	Ala	280 Tyr	Gly	Leu	Trp	Leu	285 Val	Ser	Val	Ile
	290 Glu	Val	Trp	Phe	Ala	295 Leu	Ser	Trp	Leu	Leu	300 Asp	Gln	Phe	Pro	Lys
305 Trp	Tyr	Pro	Ile	Asn	310 Arg	Glu	Thr	Tvr	Leu	315 Asp	Arg	Leu	Δla	Len	320 Arg
	Asp			325					330					335	
	Ser		340					345					350		
		355					360					365			
	Val 370					375					380		•		
385	Tyr				390					395					400
	Glu			405					410					415	_
	Asn		420					425					430	_	
	Tyr	435					440					445		_	
Ala	Met 450	Lys	Arg	Glu	Tyr	Glu 455	Glu	Phe	Lys	Ile	Arg 460	Ile	Asn	Ala	Leu
Val 465	Ala	Lys	Ala	Gln	Lys 470	Val	Pro	Glu	Glu	Gly 475	Trp	Thr	Met	Ala	Asp 480
Gly	Thr	Ala	Trp	Pro 485	Gly	Asn	Asn	Pro	Arg 490	Asp	His	Pro	Gly	Met 495	Ile
Gln	Val	Phe	Leu 500		His	Ser	Gly	Gly 505		Asp	Thr	Asp	Gly 510		Glu
Leu	Pro	Arg 515	Leu	Val	Tyr	Val	Ser 520	Arg	Glu	Lys	Arg	Pro 525	Gly	Phe	Gln
His	His 530	Lys	Lys	Ala	Gly	Ala 535		Asn	Ala	Leu	Ile 540		Val	Ser	Ala
Val 545	Leu	Thr	Asn	Gly	Ala 550		Leu	Leu	Asn	Val 555		Cys	Asp	His	Tyr 560
	Asn	Ser	Ser	Lys 565		Leu	Arg	Glu	Ala 570		Cys	Phe	Met	Met 575	
Pro	Ala	Leu	Gly 580		Lys	Thr	Cys	Tyr 585		Gln	Phe	Pro	Gln 590		Phe
Asp	Gly	Ile		Leu	His	Asp	Arg		Ala	Asn	Arg	Asn		Val	Phe

- 31 -

Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala Leu Tyr Gly Tyr Asp Pro Val Leu Thr Glu Ala Asp Leu Glu Pro Asn Ile Val Val Lys Ser Cys Cys Gly Arg Arg Lys Arg Lys Asn Lys Ser Tyr Met Asp Ser Gln Ser Arg Ile Met Lys Arg Thr Glu Ser Ser Ala Pro Ile Phe Asn Met Glu Asp Ile Glu Gly Ile Glu Gly Tyr Glu Asp Glu Arg Ser Val Leu Met Ser Gln Arg Lys Leu Glu Lys Arg Phe Gly Gln Ser Pro Ile Phe Ile Ala Ser Thr Phe Met Thr Gln Gly Gly Ile Pro Pro Ser Thr Asn Pro Ala Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Ala Arg Gly Trp Gln Ser Ile Tyr Cys Met Pro Pro Arg Pro Cys Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Val Glu Ile Leu Leu Ser Arg His Cys Pro Ile Trp Tyr Gly Tyr Asn Gly Arg Leu Lys Leu Leu Glu Arg Leu Ala Tyr Ile Asn Thr Ile Val Tyr Pro Ile Thr Ser Val Pro Leu Ile Ala Tyr Cys Val Leu Pro Ala Ile Cys Leu Leu Thr Asn Lys Phe Ile Ile Pro Glu Ile Ser Asn Tyr Ala Gly Met Phe Phe Ile Leu Leu Phe Ala Ser Ile Phe Ala Thr Gly Ile Leu Glu Leu Arg Trp Ser Gly Val Gly Ile Glu Asp Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Thr Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu Asp Gly Asp Phe Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser Leu Leu Ile Pro Pro Thr Thr Val Leu Val Ile Asn Leu Val Gly Met Val Ala Gly Ile Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ser Ile Trp Val Ile Leu His Leu Tyr Pro Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys Ile Asp Pro Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala Leu Gly Gln Cys Gly Val

WO 00/09706 PCT/US99/18760

- 32 -

```
1060
                                 1065
                                                     1070
Asn Cys
       <210> 15
       <211> 25
       <212> DNA
       <213> Zea mays
       <400> 15
atggcggcca acaaggggat ggtgg
25
       <210> 16
       <211> 25
       <212> DNA
       <213> Zea mays
       <400> 16
tcagcagttc acaccacatt gcccc
25
       <210> 17
       <211> 3969
       <212> DNA
       <213> Zea mays
       <220>
       <221> CDS
       <222> (144)...(3399)
       <400> 17
etteteete gteggtgegg egtggegegg eteggegtte ggtgagaaac eaeteggggg
                                                                       60
atgaggatet getgetagag tgagaggage taeggteagt atcetetgee ttegteggeg
                                                                       120
geggaagtgg aggggaggaa geg atg gag geg age gee ggg etg gtg gee gge
                           Met Glu Ala Ser Ala Gly Leu Val Ala Gly
tcc cac aac cgc aac gag ctc gtc gtc atc cgc cgc gac ggc gat ccc
                                                                       221
Ser His Asn Arg Asn Glu Leu Val Val Ile Arg Arg Asp Gly Asp Pro
                  15
ggg ccg aag ccg ccg cgg gag cag aac ggg cag gtg tgc cag att tgc
                                                                       269
Gly Pro Lys Pro Pro Arg Glu Gln Asn Gly Gln Val Cys Gln Ile Cys
              30
ggc gac gac gtc ggc ctt gcc ccc ggc ggg gac ccc ttc gtg gcg tgc
                                                                       317
Gly Asp Asp Val Gly Leu Ala Pro Gly Gly Asp Pro Phe Val Ala Cys
          45
                              50
                                                                       365
aac gag tgc gcc ttc ccc gtc tgc cgg gac tgc tac gaa tac gag cgc
Asn Glu Cys Ala Phe Pro Val Cys Arg Asp Cys Tyr Glu Tyr Glu Arg
      60
                          65
                                              70
egg gag ggc acg cag aac tgc ccc cag tgc aag act cga tac aag cgc
                                                                       413
Arg Glu Gly Thr Gln Asn Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg
 75
                      80
                                          85
```

WO 00/09706

ctc Leu	aag Lys	ggc	tgc Cys	caa Gln ,95	cgt Arg	gtg Val	acc Thr	ggt Gly	gac Asp 100	gag Glu	gag Glu	gag Glu	gac Asp	ggc Gly 105	gtc Val	461
					gag Glu											509
					ctc Leu											557
					cca Pro											605
ctc Leu 155	ctc Leu	acc Thr	aac Asn	GJ À aaa	caa Gln 160	atg Met	gtg Val	gat Asp	gac Asp	atc Ile 165	cca Pro	ccg Pro	gag Glu	cag Gln	cac His 170	653
gcg Ala	ctg Leu	gtg Val	cct Pro	tct Ser 175	ttc Phe	atg Met	ggt Gly	ggt Gly	180 GJA 333	gga Gly	aag Lys	agg Arg	ata Ile	cat His 185	ecc Pro	701
					ccc Pro											749
cca Pro	tcc Ser	aag Lys 205	gat Asp	ctt Leu	gct Ala	gca Ala	tat Tyr 210	gly aaa	tat Tyr	ggt Gly	agt Ser	gtt Val 215	gct Ala	tgg Trp	aag Lys	797
					tgg Trp											845
					ggt Gly 240											893
					caa Gln											941
					tat Tyr		_								_	989
					cac His			_	_		-			_	_	1037
		_			ata Ile		_		_	_				_	_	1085
				-	caa Gln			_						_		1133

- 34 -

									-								
315					320					325					330		
													ggc Gly			118	31
													gat Asp 360			122	29
													atc Ile			127	17
													gat Asp			132	25
													gaa Glu			137	13
													cct Pro			142	!1
													gac Asp 440			146	;9
													gag Glu			151	.7
													cag Gln			156	5
		Glu	Gly	Trp		Met	Gln	Asp	Gly	Thr	Pro	${\tt Trp}$	cct Pro	Gly	Asn	161	.3
													ggc Gly			166	:1
													gtt Val 520			170	19
	_			_							_		gct Ala		_	175	;7
_		_	_	_	_	_		_	_				gct Ala			180)5

WO 00/09706 PCT/US99/18760

- 35 -

								-	35 -							
ttg Leu 555	Leu	aac Asn	ttg Lev	gat Asp	tgt Cys 560	Asp	cac	tac Tyr	atc Ile	aac Asn 565	Asn	agc Ser	aag Lys	gct Ala	ata Ile 570	1853
aag Lys	gaa Glu	gca Ala	atg Met	tgt Cys 575	Phe	atg Met	atg Met	gac Asp	Pro 580	tta Leu	cta Leu	gga Gly	aag Lys	aag Lys 585	gtt Val	1901
tgc Cys	tat Tyr	gta Val	cag Gln 590	Phe	cct Pro	caa Gln	aga Arg	ttt Phe 595	Asp	gly aaa	att Ile	gat Asp	cgc Arg 600	His	gac Asp	1949
cga Arg	tat Tyr	gct Ala 605	aac Asn	cgg Arg	aat Asn	gtt Val	gtc Val 610	ttt Phe	ttt Phe	gat Asp	atc Ile	aac Asn 615	atg Met	aaa Lys	ggt Gly	1997
ttg Leu	gat Asp 620	Gly	att Ile	cag Gln	ggt Gly	cca Pro 625	att Ile	tat Tyr	gtt Val	ggt Gly	act Thr 630	gga Gly	tgt Cys	gta Val	ttt Phe	2045
aga Arg 635	agg Arg	cag Gln	gca Ala	tta Leu	tat Tyr 640	ggt Gly	tat Tyr	gat Asp	gcc Ala	ccc Pro 645	aaa Lys	aca Thr	aag Lys	aag Lys	cca Pro 650	2093
cca Pro	tca Ser	agg Arg	act Thr	tgc Cys 655	aac Asn	tgc Cys	tgg Trp	ccc Pro	aag Lys 660	tgg Trp	tgc Cys	ttt Phe	tgc Cys	tgt Cys 665	tgc Cys	2141
tgc Cys	ttt Phe	ggc	aat Asn 670	agg Arg	aag Lys	caa Gln	aag Lys	aag Lys 675	act Thr	acc Thr	aaa Lys	ccc Pro	aaa Lys 680	aca Thr	gag Glu	2189
aag Lys	aaa Lys	aag Lys 685	tta Leu	tta Leu	ttt Phe	ttc Phe	aag Lys 690	aaa Lys	gaa Glu	gag Glu	aac Asn	caa Gln 695	tcc Ser	cct Pro	gca Ala	2237
tat Tyr	gct Ala 700	ctt Leu	ggt Gly	gaa Glu	att Ile	gac Asp 705	gaa Glu	gct Ala	gct Ala	cca Pro	gga Gly 710	gct Ala	gag Glu	aat Asn	gaa Glu	2285
aag Lys 715	gcc Ala	ggt Gly	att Ile	gta Val	aat Asn 720	caa Gln	caa Gln	aaa Lys	tta Leu	gaa Glu 725	aag Lys	aaa Lys	ttt Phe	ggc Gly	caa Gln 730	2333
tct Ser	tct Ser	gtt Val	ttt Phe	gtt Val 735	aca Thr	tcc Ser	aca Thr	ctt Leu	ctc Leu 740	gag Glu	aat Asn	ggt Gly	gga Gly	acc Thr 745	ttg Leu	2381
aag Lys	agt Ser	gca Ala	agt Ser 750	cct Pro	gct Ala	tct Ser	ctt Leu	ttg Leu 755	aaa Lys	gaa Glu	gct Ala	ata Ile	cat His 760	gtc Val	att Ile	2429
agt Ser	tgt Cys	ggt Gly 765	tat Tyr	gaa Glu	gac Asp	ГÀз	aca Thr 770	gac Asp	tgg Trp	gga Gly	aaa Lys	gag Glu 775	att Ile	ggc Gly	tgg Trp	2477
atc Ile	tat Tyr	gga Gly	tca Ser	gtt Val	aca Thr	gaa Glu	gat Asp	att Ile	cta Leu	act Thr	ggt Gly	ttc Phe	aag Lys	atg Met	cat His	2525

- 36 -

	780					785					790					
tgt Cys 795	Hịs	ggt Gly	tgg Trp	cgg Arg	tca Ser 800	att Ile	tac Tyr	tgc Cys	ata Ile	cct Pro 805	aaa Lys	cgg Arg	gtt Val	gca Ala	ttc Phe 810	2573
aaa Lys	ggt Gly	tct Ser	gca Ala	cct Pro 815	ctg Leu	aat Asn	ctt Leu	tca Ser	gat Asp 820	cgt Arg	ctt Leu	cac His	cag Gln	gtg Val 825	ctt Leu	2621
cgg Arg	tgg Trp	gct Ala	ctt Leu 830	gly ggg	tct Ser	att Ile	gag Glu	atc Ile 835	ttc Phe	ttc Phe	agc Ser	aat Asn	cat His 840	tgc Cys	cct Pro	2669
ctt Leu	tgg Trp	tat Tyr 845	GJA aaa	tat Tyr	ggt Gly	ggc Gly	ggt Gly 850	ctg Leu	aaa Lys	ttt Phe	ttg Leu	gaa Glu 855	aga Arg	ttt Phe	tcc Ser	2717
			tcc Ser													2765
tac Tyr 875	tgt Cys	aca Thr	ttg Leu	cct Pro	gcc Ala 880	atc Ile	tgt Cys	tta Leu	ttg Leu	aca Thr 885	Gly ggg	aaa Lys	ttt Phe	atc Ile	act Thr 890	2813
cca Pro	gag Glu	ctg Leu	aat Asn	aat Asn 895	gtt Val	gcc Ala	agc Ser	ctg Leu	tgg Trp 900	ttc Phe	atg Met	tca Ser	ctt Leu	ttt Phe 905	atc Ile	2861
tgc Cys	att Ile	ttt Phe	gct Ala 910	acg Thr	agc Ser	atc Ile	cta Leu	gaa Glu 915	atg Met	aga Arg	tgg Trp	agt Ser	ggt Gly 920	gtt Val	gga Gly	2909
att Ile	gat Asp	gac Asp 925	tgg Trp	tgg Trp	agg Arg	aat Asn	gag Glu 930	cag Gln	ttc Phe	tgg Trp	gtc Val	att Ile 935	gga Gly	ggt Gly	gtg Val	2957
tcc Ser	tca Ser 940	cac His	ctc Leu	ttt Phe	gct Ala	gtg Val 945	ttc Phe	cag Gln	gga Gly	ctt Leu	ctc Leu 950	aag Lys	gtc Val	ata Ile	gct Ala	3005
			aca Thr													3053
			gag Glu													3101
			ttg Leu 990	Leu					Ile					Gly		3149
			atc Ile					Glu					Leu			3197

aag									37 -							
гЛs	tev Lev 102	. Phe	ttt Phe	gca Ala	ttt Phe	tgg Trp 102	Val	Ile	gtc Val	cat His	Ctt Leu 103	Tyr	ccc Pro	ttt Phe	ctc Leu	3245
Lys 103	Gly	Leu	. Val	Gly	Arg 104	Gln 0	Asn	Arg	Thr	Pro 104	Thr 5	Ile	Val	Ile	gtc Val 1050	3293
Trp) Ser	·Ile		Leu 105	Ala 5	Ser	Ile	Phe	Ser 106	Leu 0	Leu	Trp	Val	Arg 106	Ile 5	3341
Asp	Pro	Phe	107	Ala O	Lys	Asp	Asp	Gly 107	Pro 5	Leu	Leu	Glu	Glu 108	Cys 0	ggt Gly	3389
ttg Leu	gat Asp	tgc Cys 108		ctag	gatgi	t ca	gtgc	atca	gct	cccc	caa	tctg	cata	tg		3439
ggg gtg agc cag ttg	gtcc aaaa tttg aagg gaat gagg atag	caa tgg att tat gca gct tta	gttte agget gtgea tttga tetge tgtte	ettti egege agcai attei ecagi eatta agta	eg at ge at et	tccar atccr actgo actgo acag acag	tgt: tac: tcc: gage: gtc:	g aag g cag t tgg c gtg a acg t atg	cctadetgg gtcgd gtacd ctgcd actad gttt	ctta ggcc caat aaac acat gaaa tttg	atar gtgr atar ttgr tatr aaac ttgr	tetga gaata gatga gtte ttata cagaa tgta	aga ga ggc ggc tca gta ata	gata gcat tgag ataa tgcc ttag	acatga tactgg atgcaa ccgaac ggcagg tgttca cattaa catctg	3499 3559 3619 3679 3739 3799 3859 3919 3969
		210>	18													
	<:	212>	1086 PRT Zea		;											
	<: <:	212> 213> 400>	PRT Zea 18	mays												
1	<i <i <i Glu</i </i </i 	212> 213> 400> Ala	PRT Zea 18 Ser	mays Ala 5	Gly				10					15		
1	<i <i <i Glu</i </i </i 	212> 213> 400> Ala	PRT Zea 18	mays Ala 5	Gly				10				Pro	15		
1 Leu	<pre><pre><pre><pre><pre><pre><pre><pre></pre></pre></pre></pre></pre></pre></pre></pre>	212> 213> 400> Ala Val	PRT Zea 18 Ser Ile	mays Ala 5 Arg	Gly Arg	qeA	Gly	Asp 25	10 Pro	Gly	Pro	Lys Asp	Pro	15 Pro	Arg	
1 Leu Glu	<pre><pre><pre><pre><pre><pre><pre><pre></pre></pre></pre></pre></pre></pre></pre></pre>	212> 213> 400> Ala Val Asn 35	PRT Zea 18 Ser Ile 20	Mays Ala 5 Arg Gln	Gly Arg Val	Asp Cys	Gly Gln 40	Asp 25 Ile	10 Pro Cys	Gly Gly	Pro Asp Glu	Lys Asp 45	Pro 30 Val	15 Pro Gly	Arg Leu	
1 Leu Glu Ala	Glu Val Gln Pro	212> 213> 400> Ala Val Asn 35 Gly	PRT Zea 18 Ser Ile 20 Gly	Mays Ala 5 Arg Gln Asp	Gly Arg Val Pro	Asp Cys Phe 55	Gly Gln 40 Val	Asp 25 Ile Ala	10 Pro Cys Cys	Gly Gly Asn Arg	Pro Asp Glu 60	Lys Asp 45 Cys	Pro 30 Val Ala	15 Pro Gly Phe	Arg Leu Pro Asn	
1 Leu Glu Ala Val 65	Glu Val Gln Pro 50 Cys	212> 213> 400> Ala Val Asn 35 Gly	PRT Zea 18 Ser Ile 20 Gly Gly Asp Cys	Mays Ala 5 Arg Gln Asp Cys	Gly Arg Val Pro Tyr 70	Asp Cys Phe 55 Glu	Gly Gln 40 Val Tyr	Asp 25 Ile Ala Glu	10 Pro Cys Cys Arg	Gly Gly Asn Arg 75	Pro Asp Glu 60 Glu	Lys Asp 45 Cys Gly	Pro 30 Val Ala Thr	15 Pro Gly Phe Gln Gln	Arg Leu Pro Asn 80	
1 Leu Glu Ala Val 65 Cys	Glu Val Gln Pro 50 Cys	212> 213> 400> Ala Val Asn 35 Gly Arg	PRT Zea 18 Ser Ile 20 Gly Gly Asp Cys	Mays Ala 5 Arg Gln Asp Cys Lys 85	Gly Arg Val Pro Tyr 70 Thr	Asp Cys Phe 55 Glu Arg	Gly Gln 40 Val Tyr	Asp 25 Ile Ala Glu Lys Gly	10 Pro Cys Cys Arg Arg	Gly Gly Asn Arg 75 Leu	Pro Asp Glu 60 Glu Lys	Lys Asp 45 Cys Gly	Pro 30 Val Ala Thr Cys	15 Pro Gly Phe Gln Gln 95	Arg Leu Pro Asn 80 Arg	
Leu Glu Ala Val 65 Cys	Glu Val Gln Pro 50 Cys Pro	212> 213> 400> Ala Val Asn 35 Gly Arg Gln	PRT Zea 18 Ser Ile 20 Gly Gly Asp Cys	Mays Ala 5 Arg Gln Asp Cys Lys 85 Glu	Gly Arg Val Pro Tyr 70 Thr	Asp Cys Phe 55 Glu Arg Glu	Gly Gln 40 Val Tyr Tyr Asp	Asp 25 Ile Ala Glu Lys Gly 105	10 Pro Cys Cys Arg Arg 90 Val	Gly Gly Asn Arg 75 Leu Asp	Pro Asp Glu 60 Glu Lys Asp	Lys Asp 45 Cys Gly Gly Leu Glu	Pro 30 Val Ala Thr Cys Asp	15 Pro Gly Phe Gln 95 Asn	Arg Leu Pro Asn 80 Arg	
Leu Glu Ala Val 65 Cys Val Phe	Glu Val Gln Pro 50 Cys Pro Thr	212> 213> 400> Ala Val Asn 35 Gly Arg Gln Gly Trp 115	PRT Zea 18 Ser Ile 20 Gly Gly Asp Cys Asp	Mays Ala 5 Arg Gln Asp Cys Lys 85 Glu Gly	Gly Arg Val Pro Tyr 70 Thr Glu His	Asp Cys Phe 55 Glu Arg Glu Asp	Gly Gln 40 Val Tyr Tyr Asp Ser 120	Asp 25 Ile Ala Glu Lys Gly 105 Gln	10 Pro Cys Cys Arg Arg 90 Val	Gly Gly Asn Arg 75 Leu Asp Val	Pro Asp Glu 60 Glu Lys Asp Ala Pro	Lys Asp 45 Cys Gly Gly Leu Glu 125	Pro 30 Val Ala Thr Cys Asp 110 Ser	15 Pro Gly Phe Gln Gln 95 Asn	Arg Leu Pro Asn 80 Arg Glu Leu	
Leu Glu Ala Val 65 Cys Val Phe Tyr Gln	Glu Val Gln Pro 50 Cys Pro Thr Asn Gly 130	212> 213> 400> Ala Val Asn 35 Gly Arg Gln Gly Trp 115 His	PRT Zea 18 Ser Ile 20 Gly Gly Asp 100 Asp	Mays Ala 5 Arg Gln Asp Cys Lys 85 Glu Gly Ser Leu	Gly Arg Val Pro Tyr 70 Thr Glu His	Asp Cys Phe 55 Glu Arg Glu Asp Gly 135	Gly Gln 40 Val Tyr Tyr Asp Ser 120 Arg	Asp 25 Ile Ala Glu Lys Gly 105 Gln	10 Pro Cys Cys Arg Arg 90 Val Ser	Gly Gly Asn Arg 75 Leu Asp Val Asp Leu	Pro Asp Glu 60 Glu Lys Asp Ala Pro 140	Lys Asp 45 Cys Gly Gly Leu Glu 125 Asn	Pro 30 Val Ala Thr Cys Asp 110 Ser	15 Pro Gly Phe Gln Gln 95 Asn Met	Arg Leu Pro Asn 80 Arg Glu Leu Pro Gln	
Leu Glu Ala Val 65 Cys Val Phe Tyr Gln 145	Glu Val Gln Pro 50 Cys Pro Thr Asn Gly 130 Ala	212> 213> 400> Ala Val Asn 35 Gly Arg Gln Gly Trp 115 His	PRT Zea 18 Ser Ile 20 Gly Gly Asp Cys Asp 100 Asp	Mays Ala 5 Arg Gln Asp Cys Lys 85 Glu Gly Ser Leu	Gly Arg Val Pro Tyr 70 Thr Glu His Tyr Asn	Asp Cys Phe 55 Glu Arg Glu Asp Gly 135 Pro	Gly Gln 40 Val Tyr Tyr Asp Ser 120 Arg	Asp 25 Ile Ala Glu Lys Gly 105 Gln Gly Val	10 Pro Cys Cys Arg Arg 90 Val Ser Gly	Gly Gly Asn Arg 75 Leu Asp Val Asp Leu 155	Pro Asp Glu 60 Glu Lys Asp Ala Pro 140 Leu	Lys Asp 45 Cys Gly Gly Leu Glu 125 Asn	Pro 30 Val Ala Thr Cys Asp 110 Ser Gly	15 Pro Gly Phe Gln 95 Asn Met Ala	Arg Leu Pro Asn 80 Arg Glu Leu Pro Gln 160	

Met Gly Gly Gly Lys Arg Ile His Pro Leu Pro Tyr Ala Asp Pro 185 Ser Leu Pro Val Gln Pro Arg Ser Met Asp Pro Ser Lys Asp Leu Ala 200 Ala Tyr Gly Tyr Gly Ser Val Ala Trp Lys Glu Arg Met Glu Asn Trp Lys Gln Arg Gln Glu Arg Met His Gln Thr Gly Asn Asp Gly Gly 235 Asp Asp Gly Asp Asp Ala Asp Leu Pro Leu Met Asp Glu Ala Arg Gln 250 Gln Leu Ser Arg Lys Ile Pro Leu Pro Ser Ser Gln Ile Asn Pro Tyr 265 Arg Met Ile Ile Ile Arg Leu Val Val Leu Gly Phe Phe His 280 Tyr Arg Val Met His Pro Val Asn Asp Ala Phe Ala Leu Trp Leu Ile 295 300 Ser Val Ile Cys Glu Ile Trp Phe Ala Met Ser Trp Ile Leu Asp Gln 310 315 Phe Pro Lys Trp Phe Pro Ile Glu Arg Glu Thr Tyr Leu Asp Arg Leu 325 330 Ser Leu Arg Phe Asp Lys Glu Gly Gln Pro Ser Gln Leu Ala Pro Ile 345 Asp Phe Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro Leu Val 360 Thr Thr Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro Val Asp 375 380 Lys Val Ser Cys Tyr Val Ser Asp Gly Ala Ala Met Leu Thr Phe 390 395 Glu Ala Leu Ser Glu Thr Ser Glu Phe Ala Lys Lys Trp Val Pro Phe 405 410 Cys Lys Arg Tyr Asn Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe Gln 420 425 Gln Lys Ile Asp Tyr Leu Lys Asp Lys Val Ala Ala Asn Phe Val Arg 440 Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile 455 460 Asn Ala Leu Val Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr 470 475 Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val Arg Asp His Pro 485 490 Gly Met Ile Gln Val Phe Leu Gly Gln Ser Gly Gly Leu Asp Cys Glu 505 Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro 520 Gly Tyr Asn His His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg 535 540 Val Ser Ala Val Leu Thr Asn Ala Pro Tyr Leu Leu Asn Leu Asp Cys 550 555 Asp His Tyr Ile Asn Asn Ser Lys Ala Ile Lys Glu Ala Met Cys Phe 570 Met Met Asp Pro Leu Leu Gly Lys Lys Val Cys Tyr Val Gln Phe Pro 585 Gln Arg Phe Asp Gly Ile Asp Arg His Asp Arg Tyr Ala Asn Arg Asn 600 Val Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly 620 Pro Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Arg Gln Ala Leu Tyr 625 € 635

Gly Tyr Asp Ala Pro Lys Thr Lys Lys Pro Pro Ser Arg Thr Cys Asn 645 650 Cys Trp Pro Lys Trp Cys Phe Cys Cys Cys Phe Gly Asn Arg Lys 665 Gln Lys Lys Thr Thr Lys Pro Lys Thr Glu Lys Lys Leu Leu Phe 680 Phe Lys Lys Glu Glu Asn Gln Ser Pro Ala Tyr Ala Leu Gly Glu Ile 695 700 Asp Glu Ala Ala Pro Gly Ala Glu Asn Glu Lys Ala Gly Ile Val Asn 710 715 Gln Gln Lys Leu Glu Lys Lys Phe Gly Gln Ser Ser Val Phe Val Thr 730 Ser Thr Leu Leu Glu Asn Gly Gly Thr Leu Lys Ser Ala Ser Pro Ala 745 Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp 760 Lys Thr Asp Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr 775 Glu Asp Ile Leu Thr Gly Phe Lys Met His Cys His Gly Trp Arg Ser 790 795 Ile Tyr Cys Ile Pro Lys Arg Val Ala Phe Lys Gly Ser Ala Pro Leu 810 Asn Leu Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser 825 Ile Glu Ile Phe Phe Ser Asn His Cys Pro Leu Trp Tyr Gly Tyr Gly 840 Gly Gly Leu Lys Phe Leu Glu Arg Phe Ser Tyr Ile Asn Ser Ile Val 855 Tyr Pro Trp Thr Ser Ile Pro Leu Leu Ala Tyr Cys Thr Leu Pro Ala 870 Ile Cys Leu Leu Thr Gly Lys Phe Ile Thr Pro Glu Leu Asn Asn Val 885 890 Ala Ser Leu Trp Phe Met Ser Leu Phe Ile Cys Ile Phe Ala Thr Ser 900 905 Ile Leu Glu Met Arg Trp Ser Gly Val Gly Ile Asp Asp Trp Trp Arg 920 925 Asn Glu Gln Phe Trp Val Ile Gly Gly Val Ser Ser His Leu Phe Ala 935 940 Val Phe Gln Gly Leu Leu Lys Val Ile Ala Gly Val Asp Thr Ser Phe 950 955 Thr Val Thr Ser Lys Gly Gly Asp Asp Glu Glu Phe Ser Glu Leu Tyr 965 970 Thr Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Leu 980 985 Leu Asn Phe Ile Gly Val Val Ala Gly Val Ser Asn Ala Ile Asn Asn 995 1000 Gly Tyr Glu Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe 1015 1020 Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys Gly Leu Val Gly Arg 1030 1035 Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp Ser Ile Leu Leu Ala 1045 1050 Ser Ile Phe Ser Leu Leu Trp Val Arg Ile Asp Pro Phe Leu Ala Lys 1065 Asp Asp Gly Pro Leu Leu Glu Glu Cys Gly Leu Asp Cys Asn 1075 1080

```
<211> 25
      <212> DNA
      <213> Zea mays
      <400> 19
atggaggcga gcgccgggct ggtgg
25
      <210> 20
      <211> 25
      <212> DNA
      <213> Zea mays
      <400> 20
ctagttgcaa tccaaaccac actcc
      <210> 21
      <211> 3725
      <212> DNA.
      <213> Zea mays
      <220>
      <221> CDS
      <222> (179)...(3398)
      <400> 21
gcagcagcag caccaccact gcgcggcatt gcagcgagca agcgggaggg atctggggca
                                                                   60
tggtggcggt cgctgccgct gccgctcgga tctagagggc cgcacgggct gattgccctc
                                                                   120
cgccggcctc gtcggtgtcg gtggagtgtg aatcggtgtg tgtaggagga gcgcggag
                                                                   178
atg geg gec aac aag ggg atg gtg gea gge tet cac aac egc aac gag
                                                                   226
Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu
ttc gtc atg atc cgc cac gac ggc gac gcg cct gtc ccg gct aag ccc
                                                                   274
Phe Val Met Ile Arg His Asp Gly Asp Ala Pro Val Pro Ala Lys Pro
             20
acg aag agt gcg aat ggg cag gtc tgc cag att tgt ggc gac act gtt
Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Thr Val
        35
ggc gtt tca gcc act ggt gat gtc ttt gtt gcc tgc aat gag tgt gcc
                                                                   370
Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala
     50
                        55
ttc cct gtc tgc cgc cct tgc tat gag tac gag cgc aag gaa ggg aac
                                                                   418
Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn
65
caa tgc tgc cct cag tgc aag act aga tac aag aga cag aaa ggt agc
                                                                   466
Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly Ser
                85
                                   90
514
Pro Arg Val His Gly Asp Asp Glu Glu Glu Asp Val Asp Asp Leu Asp
           100
                               105
```

- 41 -

													•			
aat Asn	gaa Glu	ttc Phe 115	aac Asn	tat Tyr	aag Lys	caa Gln	ggc Gly 120	aat Asn	ggg	aag Lys	ggc	cca Pro 125	gag Glu	tgg Trp	cag Gln	562
ctt Leu	caa Gln 130	gga Gly	gat Asp	gac Asp	gct Ala	gat Asp 135	ctg Leu	tct Ser	tca Ser	tct Ser	gct Ala 140	cgc Arg	cat His	gac Asp	cca Pro	610
cac His 145	cat His	cgg Arg	att Ile	cca Pro	cgc Arg 150	ctt Leu	aca Thr	agt Ser	gga Gly	caa Gln 155	cag Gln	ata Ile	tcț Ser	gga Gly	gag Glu 160	658
atc Ile	cct Pro	gat Asp	gca Ala	tcc Ser 165	cct Pro	gac Asp	cgt Arg.	cat His	tct Ser 170	atc Ile	cgc Arg	agt Ser	cca Pro	aca Thr 175	tcg Ser	706
agc Ser	tat Tyr	gtt Val	gat Asp 180	cca Pro	agc Ser	gtt Val	cca Pro	gtt Val 185	cct Pro	gtg Val	agg Arg	att Ile	gtg Val 190	gac Asp	ccc Pro	754
												gac Asp 205				802
												atg Met				850
act Thr 225	aat Asn	aaa Lys	tat Tyr	cca Pro	gag Glu 230	gct Ala	aga Arg	gga Gly	gac Asp	atg Met 235	gag Glu	gly ggg	act Thr	ggc Gly	tca Ser 240	898
aat Asn	gga Gly	gaa Glu	gat Asp	atg Met 245	caa Gln	atg Met	gtt Val	gat Asp	gat Asp 250	gca Ala	cgc Arg	cta Leu	cct Pro	ttg Leu 255	agc Ser	946
												tac Tyr				994
atc Ile	att Ile	ctc Leu 275	cgt Arg	ctt Leu	atc Ile	atc Ile	ctg Leu 280	tgc Cys	ttc Phe	ttc Phe	ttc Phe	caa Gln 285	tat Tyr	cgt Arg	atc Ile	1042
												gta Val				1090
												cag Gln				1138
												ctt Leu				1186
												att Ile				1234

- 42 -

			340					345					350			
					cca Pro											1282
act Thr	gtt Val 370	ttg Leu	tcc Ser	att	ctt Leu	gct Ala 375	gtg Val	gat Asp	tac Tyr	cct Pro	gtt Val 380	gac Asp	aaa Lys	gtg Val	tca Ser	1330
					gat Asp 390											1378
					ttt Phe											1426
					aga Arg											1474
					aaa Lys											1522
					tat Tyr											1570
					aaa Lys 470											1618
					Gly ggg											1666
					cac His											1714
					tat Tyr											1762
					ggt Gly											1810
					gcc Ala 550						-	_	_			1858
					gct Ala											1906

- 43 -

cca Pro	gct	cta Leu	gga Gly 580	Arg	aaa Lys	act Thr	tgt Cys	tat Tyr 585	gta Val	caa Gln	ttt Phe	cca Pro	caa Glm 590	Arg	ttt Phe	1954
gat Asp	ggc	att Ile 595	Asp	ttg Leu	cac His	gat Asp	cga Arg 600	Tyr	gct Ala	aat Asn	agg Arg	aac Asn 605	Ile	gtc Val	ttc Phe	2002
ttt Phe	gat Asp 610	Ile	aac Asn	atg Met	aaa Lys	ggt Gly 615	Leu	gat Asp	ggc	att Ile	cag Gln 620	Gly	cca Pro	gtc Val	tat Tyr	2050
gtg Val 625	gga Gly	aca Thr	gga Gly	Cya	tgt Cys 630	ttc Phe	aat Asn	agg Arg	cag Gln	gct Ala 635	Leu	tat Tyr	gga Gly	tat	gat Asp 640	2098
cct Pro	gtt Val	ttg Leu	act Thr	gaa Glu 645	gct Ala	gat Asp	ctg Leu	gaa Glu	cct Pro 650	aac Asn	att Ile	gtt Val	gtt Val	aag Lys 655	agc Ser	2146
tgc Cys	tgt Cys	ggt Gly	aga Arg 660	agg Arg	aag Lys	aga Arg	aag Lys	aac Asn 665	aag Lys	agt Ser	tat Tyr	atg Met	gat Asp 670	agt Ser	caa Gln	2194
agc Ser	cgt Arg	att Ile 675	atg Met	aag Lys	aga Arg	aca Thr	gaa Glu 680	tct Ser	tca Ser	gct Ala	ccc Pro	atc Ile 685	ttt Phe	aac Asn	atg Met	2242
gaa Glu	gac Asp 690	atc Ile	gag Glu	gag Glu	ggt Gly	att Ile 695	gaa Glu	ggt Gly	tat Tyr	gag Glu	gat Asp 700	gaa Glu	agg Arg	tca Ser	gtg Val	2290
ctt Leu 705	atg Met	tcc Ser	cag Gln	agg Arg	aaa Lys 710	ttg Leu	gag Glu	aaa Lys	cgc Arg	ttt Phe 715	ggt Gly	cag Gln	tct Ser	cca Pro	atc Ile 720	2338
ttc Phe	att Ile	gca Ala	tcc Ser	acc Thr 725	ttt Phe	atg Met	act Thr	caa Gln	ggt Gly 730	ggc	ata Ile	cca Pro	cct Pro	tca Ser 735	aca Thr	2386
aac Asn	cca Pro	gct Ala	tct Ser 740	cta Leu	ctg Leu	aag Lys	gaa Glu	gct Ala 745	atc Ile	cat His	gtt Val	atc Ile	agc Ser 750	tgt Cys	gly aaa	2434
tac Tyr	gag Glu	gac Asp 755	aaa Lys	act Thr	gaa Glu	tgg Trp	gga Gly 760	aaa Lys	gag Glu	att Ile	ggc Gly	tgg Trp 765	atc Ile	tat Tyr	ggt Gly	2482
tca Ser	gtt Val 770	aca Thr	gag Glu	gat Asp	att Ile	ctg Leu 775	act Thr	gly ggg	ttt Phe	aaa Lys	atg Met 780	cat His	gca Ala	aga Arg	ggc Gly	2530
tgg Trp 785	caa Gln	tca Ser	atc Ile	tac Tyr	tgc Cys 790	atg Met	cca Pro	cca Pro	cga Arg	cct Pro 795	tgt Cys	ttc Phe	aag Lys	ggt Gly	tct Ser 800	2578
gca Ala	cca Pro	atc Ile	aat Asn	ctt Leu	tct Ser	gat Asp	cgt Arg	ctt Leu	aat Asn	cag Gln	gtg Val	ctc Leu	cgt Arg	tgg Trp	gct Ala	2626

- 44 -

								-								
				805					810					815		
ctt Leu	GJ y 999	tca Ser	gtg Val 820	gaa Glu	att Ile	ctg Leu	ctt Leu	agc Ser 825	aga Arg	cat His	tgt Cys	cct Pro	ata Ile 830	Trp	tat Tyr	2674
ggc	tac Tyr	aat Asn 835	gly ggg	cga Arg	ttg Leu	aag Lys	ctt Leu 840	Leu	gag Glu	agg Arg	ctg Leu	gct Ala 845	tac Tyr	att Ile	aac Asn	2722
acc Thr	att Ile 850	gtt Val	tat Tyr	cca Pro	atc Ile	aca Thr 855	tct Ser	gtt Val	ccg Pro	ctt Leu	atc Ile 860	Ala	tat Tyr	tgt Cys	gtg Val	2770
ctt Leu 865	cct Pro	gct Ala	atc Ile	tgt Cys	ctt Leu 870	ctt Leu	acc Thr	aat Asn	aaa Lys	ttt Phe 875	atc Ile	att Ile	cct Pro	gag Glu	att Ile 880	2818
agt Ser	aat Asn	tat Tyr	gct Ala	gga Gly 885	atg Met	ttc Phe	ttc Phe	att Ile	ctt Leu 890	ctt Leu	ttt Phe	gcc Ala	tcc Ser	att Ile 895	ttc Phe	2866
gca Ala	act Thr	ggt Gly	ata Ile 900	ttg Leu	gag Glu	ctc Leu	aga Arg	tgg Trp 905	agt Ser	ggt Gly	gtt Val	ggc	att Ile 910	gaa Glu	gat Asp	2914
tgg Trp	tgg Trp	aga Arg 915	aat Asn	gag Glu	cag Gln	ttt Phe	tgg Trp 920	gtt Val	att Ile	ggt Gly	ggc Gly	acc Thr 925	tct Ser	gcc Ala	cat His	2962
ctc Leu	ttc Phe 930	gcg Ala	gtg Val	ttc Phe	cag Gln	ggt Gly 935	ctg Leu	ctg Leu	aaa Lys	gtg Val	ttg Leu 940	gct Ala	gly ggg	att Ile	gat Asp	3010
acc Thr 945	aac Asn	ttc Phe	aca Thr	gtt Val	acc Thr 950	tca Ser	aag Lys	gca Ala	tct Ser	gat Asp 955	gag Glu	gat Asp	ggc Gly	gac Asp	ttt Phe 960	3058
gct Ala	gag Glu	cta Leu	tat Tyr	gtg Val 965	ttc Phe	aag Lys	tgg Trp	acc Thr	agt Ser 970	ttg Leu	ctc Leu	atc Ile	cct Pro	ccg Pro 975	acc Thr	3106
act Thr	gtt Val	ctt Leu	gtc Val 980	att Ile	aac Asn	ctg Leu	gtc Val	gga Gly 985	atg Met	gtg Val	gca Ala	gga Gly	att Ile 990	tcg Ser	tat Tyr	3154
gcc Ala	att Ile	aac Asn 995	Ser	ggc	tac Tyr	caa Gln	tcc Ser 1000	Trp	ggt Gly	ccg Pro	ctc Leu	ttt Phe 1005	Gly	aag Lys	ctg Leu	3202
Phe	ttc Phe 1010	Ser	atc Ile	tgg Trp		atc Ile 1015	Leu	cat His	ctc Leu	tac Tyr	ccc Pro 1020	Phe	ctc Leu	aag Lys	ggt Gly	3250
ctc Leu 1025	Met	ggc Gly	agg Arg	cag Gln	aac Asn 1030	Arg	acg Thr	cca Pro	aca Thr	atc Ile 1035	Val	atc Ile	gtt Val	tgg Trp	tcc Ser 1040	3298

- 45 -

atc ctc ctt gcg tct atc ttc tcc ttg ctg tgg gtg aag atc gat cct Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys Ile Asp Pro 1045 1050 1055	3346
ttc atc tcc ccg aca cag aaa gct gcc gcc ttg ggg caa tgt ggt gtg Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala Leu Gly Gln Cys Gly Val 1060 1065 1070	3394
aac t getgateeag attgtgaete ttatetgaag aggeteagee aaagatetge Asn	3448
cccctcgtgt aaatacctga gggggctaga tgggaatttt ttgttgtaga tgaggatgga tctgcatcca agttatgcct ctgtttatta gcttcttcgg tgccggtgct gctgcagaca atcatggagc ctttctacct tgcttgtagt gctggccagc agcgtaaatt gtgaattctg catttttta tacgtggtgt ttattgttt agagtaaatt atcatttgtt tgaggtaact attcacacga actatatggc aatgctgtta tttaaaa	3508 3568 3628 3688 3725
<210> 22 <211> 1074	
<212> PRT <213> Zea mays	
<400> 22	
Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu 1 5 10 15	
Phe Val Met Ile Arg His Asp Gly Asp Ala Pro Val Pro Ala Lys Pro	
Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Thr Val	
Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala 50 55 60	
Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn 65 70 75 80	
Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly Ser	
Pro Arg Val His Gly Asp Asp Glu Glu Asp Val Asp Asp Leu Asp	
Asn Glu Phe Asn Tyr Lys Gln Gly Asn Gly Lys Gly Pro Glu Trp Gln	
115 120 125 Leu Gln Gly Asp Asp Ala Asp Leu Ser Ser Ala Arg His Asp Pro	
130 135 140 His His Arg Ile Pro Arg Leu Thr Ser Gly Gln Gln Ile Ser Gly Glu	
145 150 155 160 Ile Pro Asp Ala Ser Pro Asp Arg His Ser Ile Arg Ser Pro Thr Ser	
165 170 175 Ser Tyr Val Asp Pro Ser Val Pro Val Arg Ile Val Asp Pro	
180 185 190 Ser Lys Asp Leu Asn Ser Tyr Gly Leu Asn Ser Val Asp Trp Lys Glu	
195 200 205 Arg Val Glu Ser Trp Arg Val Lys Gln Asp Lys Asn Met Leu Gln Val	
210 215 220	
Thr Asn Lys Tyr Pro Glu Ala Arg Gly Asp Met Glu Gly Thr Gly Ser 225 230 235 240	
Asn Gly Glu Asp Met Gln Met Val Asp Asp Ala Arg Leu Pro Leu Ser 245 250 255	
Arg Ile Val Pro Ile Ser Ser Asn Gln Leu Asn Leu Tyr Arg Ile Val 260 265 270	

- 46 -

Ile	Ile	Leu 275	Arg	Leu	Ile	Ile	Leu 280	Cys	Phe	Phe	Phe	Gln 285	Tyr	Arg	Ile
Ser	His 290	Pro	Val	Arg	Asn	Ala 295	Tyr	Gly	Leu	Trp	Leu 300	Val	Ser	Val	Ile
Cys 305	Glu	Val	Trp	Phe	Ala 310	Leu	Ser	Trp	Leu	Leu 315	Asp	Gln	Phe	Pro	Lys 320
Trp	Tyr	Pro	Ile	Asn 325	Arg	Glu	Thr	Tyr	Leu 330	Asp	Arg	Leu	Ala	Leu 335	Arg
Tyr	Asp	Arg	Glu 340	Gly	Glu	Pro	Ser	Gln 345	Leu	Ala	Pro	Ile	Asp 350	Val	Phe
Val	Ser	Thr 355	Val	Asp	Pro	Leu	Lys 360	Glu	Pro	Pro	Leu	Ile 365	Thr	Ala	Asn
Thr	Val 370	Leu	Ser	Ile	Leu	Ala 375	Val	Asp	Tyr	Pro	Val 380	Asp	Lys	Val	Ser
Cys 385	Tyr	Val	Ser	Asp	Asp 390	Gly	Ser	Ala	Met	Leu 395	Thr	Phe	Glu	Ser	Leu 400
Ser	Glu	Thr	Ala	Glu 405	Phe	Ala	Arg	Lys	Trp 410	Val	Pro	Phe	Суз	Lys 415	Lys
His	Asn	Ile	Glu 420	Pro	Arg	Ala	Pro	Glu 425	Phe	Tyr	Phe	Ala	Gln 430	Lys	Ile
	Tyr	435					440					445		_	•
	Met 450					455					460				
465	Ala				470					475					480
	Thr			485					490					495	
	Val		500					505					510		
	Pro	515					520					525			
	His 530				_	535					540				
545	Leu				550					555	_	_	_		560
	Asn			565					570		_			575	_
	Ala		580					585					590	_	
	Gly	595					600					605			
	Asp 610					615					620	_			=
625	Gly				630					635		_			640
	Val			645					650					655	
	Cys		660					665					670		
	Arg	675					680					685			
	Asp 690				_	695		_	-		700		_		
705	Met			-	710					715	_				720
Phe	Ile	Ala	Ser	Thr 725	Phe	Met	Thr	Gln	Gly 730	Gly	Ile	Pro	Pro	Ser 735	Thr

Asn Pro Ala Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly 745 Tyr Glu Asp Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly 760 Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Ala Arg Gly 775 780 Trp Gln Ser Ile Tyr Cys Met Pro Pro Arg Pro Cys Phe Lys Gly Ser 790 795 Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp Ala 805 810 Leu Gly Ser Val Glu Ile Leu Leu Ser Arg His Cys Pro Ile Trp Tyr 820 825 Gly Tyr Asn Gly Arg Leu Lys Leu Leu Glu Arg Leu Ala Tyr Ile Asn 835 840 Thr Ile Val Tyr Pro Ile Thr Ser Val Pro Leu Ile Ala Tyr Cys Val 855 Leu Pro Ala Ile Cys Leu Leu Thr Asn Lys Phe Ile Ile Pro Glu Ile 870 875 Ser Asn Tyr Ala Gly Met Phe Phe Ile Leu Leu Phe Ala Ser Ile Phe 885 890 Ala Thr Gly Ile Leu Glu Leu Arg Trp Ser Gly Val Gly Ile Glu Asp 900 905 Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Thr Ser Ala His 920 Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala Gly Ile Asp 935 Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu Asp Gly Asp Phe 950 955 Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser Leu Leu Ile Pro Pro Thr 965 970 Thr Val Leu Val Ile Asn Leu Val Gly Met Val Ala Gly Ile Ser Tyr 985 Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys Leu 1000 1005 Phe Phe Ser Ile Trp Val Ile Leu His Leu Tyr Pro Phe Leu Lys Gly 1015 1020 Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp Ser 1035 1030 Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys Ile Asp Pro 1045 1050 Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala Leu Gly Gln Cys Gly Val 1065 Asn Cys

<210> 23

<211> 25

<212> DNA

<213> Zea mays

<400> 23

atggcggcca acaaggggat ggtgg 25

<210> 24

<211> 25

<212> DNA

<213> Zea mays

<400> 24 tcagcagttc acaccacatt gcccc 25

<210> 25
<211> 3813
<212> DNA
<213> Zea mays

<220>
<221> CDS
<222> (215)...(3494)

<222> (215)(3494)	
<pre><400> 25 ccacagctca tataccaaga gccggagcag cttagcgcag cccagagcgg cgccgcgcca agcacaaccc ccacccgcca cagccgcgtg cgcatgtgag cggtcgccgc ggccgggaga ccagaggagg ggaggactac gtgcatttcg ctgtgccgcc gccgcggggt tcgtgcgcga gcgagatccg gcggggggg gcggggggcc tgag atg gag gct agc gcg ggg ctg</pre>	60 120 180 235
gtg gcc ggc tcg cat aac cgg aac gag ctg gtg gtg atc cgc cgc gac Val Ala Gly Ser His Asn Arg Asn Glu Leu Val Val Ile Arg Arg Asp 10 15 20	283
cgc gag tcg gga gcc gcg ggc ggc gcg gcg cgc cgg gcg gag gcg Arg Glu Ser Gly Ala Ala Gly Gly Gly Ala Ala Arg Arg Ala Glu Ala 25 30 35	331
ccg tgc cag ata tgc ggc gac gag gtc ggg gtg ggc ttc gac ggg gag Pro Cys Gln Ile Cys Gly Asp Glu Val Gly Val Gly Phe Asp Gly Glu 40 45 50 55	379
ccc ttc gtg gcg tgc aac gag tgc gcc ttc ccc gtc tgc cgc gcc tgc Pro Phe Val Ala Cys Asn Glu Cys Ala Phe Pro Val Cys Arg Ala Cys 60 65 70	427
tac gag tac gag cgc cgc gag ggc tcg caa gcg tgc ccg cag tgc agg Tyr Glu Tyr Glu Arg Arg Glu Gly Ser Gln Ala Cys Pro Gln Cys Arg 75 80 85	475
acc cgc tac aag cgc ctc aag ggc tgc ccg cgg gtg gcc ggc gac gag Thr Arg Tyr Lys Arg Leu Lys Gly Cys Pro Arg Val Ala Gly Asp Glu 90 95 100	523
gag gag gac ggc gtc gac gac ctg gag ggc gag ttc ggc ctg cag gac Glu Glu Asp Gly Val Asp Asp Leu Glu Gly Glu Phe Gly Leu Gln Asp 105 110 115	571
ggc gcc gcc cac gag gac gac ccg cag tac gtc gcc gag tcc atg ctc Gly Ala Ala His Glu Asp Asp Pro Gln Tyr Val Ala Glu Ser Met Leu 120 125 130 135	619
agg gcg cag atg agc tac ggc cgc ggc ggc gac gcg cac ccc ggc ttc	667

Arg Ala Gln Met Ser Tyr Gly Arg Gly Gly Asp Ala His Pro Gly Phe

- 49 -

ago Ser	Pro	gto Val	pro	Asr	gtg Val	ccg Pro	ctc Leu	ctc Leu 160	Thr	aac Asn	gly Gly	cag Gln	atg Met	Val	gat Asp	715
gac Asp	ato Ile	e ccg Pro 170	Pro	gag Glu	cag Gln	cac His	gcg Ala 175	Leu	gtg Val	ccg Pro	tcc Ser	tac Tyr 180	Met	ago Ser	ggc	763
Gly	ggc Gly 185	GLY	gly	ggc	aag Lys	agg Arg 190	atc Ile	cac His	ccg Pro	ctc Leu	Pro	Phe	gca Ala	gat Asp	ccc Pro	811
200	. Leu	Pro	Val	Gln	Pro 205	Arg	Ser	Met	Asp	Pro 210	Ser	aag Lys	Asp	Leu	Ala 215	859
Ala	Tyr	GIA	Tyr	Gly 220	Ser	Val	Ala	Trp	Lys 225	Glu	Arg	atg Met	Glu	Gly 230	Trp	907
rys	Gin	Lys	G1n 235	Glu	Arg	Leu	Gln	His 240	Val	Arg	Ser	gag Glu	Gly 245	Gly	Gly	955
Asp	Trp	Asp 250	Gly	Asp	Ąsp	Ala	Asp 255	Leu	Pro	Leu	Met	gat Asp 260	Glu	Ala	Arg	1003
GIn	Pro 265	Leu	Ser	Arg	Lys	Val 270	Pro	Ile	Ser	Ser	Ser 275	cga Arg	Ile	Asn	Pro	1051
1yr 280	Arg	Met	Ile	Ile	Val 285	Ile	Arg	Leu	Val	Val 290	Leu	ggt Gly	Phe	Phe	Phe 295	1099
His	Tyr	Arg	Val	Met 300	His	Pro	Ala	Lys	Asp 305	Ala	Phe	gca Ala	Leu	Trp 310	Leu	1147
Ile	Ser	Val	Ile 315	Сув	Glu	Ile	Trp	Phe 320	Ala	Met	Ser	tgg Trp	Ile 325	Leu	Asp	1195
cag Gln	ttc Phe	cca Pro 330	aag Lys	tgg Trp	ctt Leu	Pro	atc Ile 335	gag Glu	aga Arg	gag Glu	act Thr	tac Tyr 340	ctg Leu	gac Asp	cgt Arg	1243
ttg Leu	tca Ser 345	cta Leu	agg Arg	ttt Phe	gac Asp	aag Lys 350	gaa Glu	ggt Gly	caa Gln	ccc Pro	tct Ser 355	cag Gln	ctt Leu	gct Ala	cca Pro	1291
atc Ile 360	gac Asp	ttc Phe	ttt Phe	gtc Val	agt Ser 365	acg Thr	gtt Val	gat Asp	Pro	aca Thr 370	aag Lys	gaa Glu	cct Pro	ccc Pro	ttg Leu 375	1339
gtc Val	aca Thr	gcg Ala	aac Asn	act Thr	gtc Val	ctt Leu	tcc Ser	atc Ile	ctt Leu	tct Ser	gtg Val	gat Asp	tat Tyr	ccg Pro	gtt Val	1387

- 50 -

380		385	390
gag aag gtc tcc tgc Glu Lys Val Ser Cys 395	tat gtt tct gat Tyr Val Ser Asp 400	gat ggt gct gca atg Asp Gly Ala Ala Met 405	Leu Thr
ttt gaa gca ttg tct Phe Glu Ala Leu Ser 410	gaa aca tct gaa Glu Thr Ser Glu 415	ttt gca aag aaa tgg Phe Ala Lys Lys Trp 420	gtt cct 1483 Val Pro
ttc agc aaa aag ttt Phe Ser Lys Lys Phe 425	aat atc gag cct Asn Ile Glu Pro 430	cgt gct cct gag tgg Arg Ala Pro Glu Trp 435	tac ttc 1531 Tyr Phe
caa cag aag ata gac Gln Gln Lys Ile Asp 440	tac ctg aaa gac Tyr Leu Lys Asp 445	aag gtt gct gct tca Lys Val Ala Ala Ser 450	ttt gtt 1579 Phe Val 455
agg gag agg agg gcg Arg Glu Arg Arg Ala 460	atg aag aga gaa Met Lys Arg Glu	tac gag gaa ttc aag Tyr Glu Glu Phe Lys 465	gta agg 1627 Val Arg 470
atc aat gcc ttg gtt : Ile Asn Ala Leu Val : 475	gca aaa gcc caa Ala Lys Ala Gln 480	aag gtt cct gag gaa Lys Val Pro Glu Glu 485	gga tgg 1675 Gly Trp
aca atg caa gat gga Thr Met Gln Asp Gly : 490	agc ccc tgg cct Ser Pro Trp Pro 495	gga aac aac gta cgc Gly Asn Asn Val Arg 500	gat cat 1723 Asp His
cct gga atg att cag g Pro Gly Met Ile Gln 7 505	gta ttc ctt ggc Val Phe Leu Gly 510	caa agt ggc ggt cgt Gln Ser Gly Gly Arg 515	gat gtg 1771 Asp Val
gaa gga aat gag ttg Glu Gly Asn Glu Leu 520	cct cgc ctg gtt Pro Arg Leu Val 525	tat gtc tcg aga gaa Tyr Val Ser Arg Glu 530	aag agg 1819 Lys Arg 535
cca ggt tat aac cat o Pro Gly Tyr Asn His 1 540	His Lys Lys Ala	ggt gcc atg aat gca Gly Ala Met Asn Ala 545	ctg gtc 1867 Leu Val 550
egt gte tet get gte t Arg Val Ser Ala Val 1 555			
tgt gat cac tac atc a Cys Asp His Tyr Ile i 570			
ttc atg atg gat cct to the Met Met Asp Pro 1 585			
cct cag agg ttt gat g Pro Gln Arg Phe Asp 6			

WO 00/09706

	aac Asn	gtt Val	gtc Val	ttt Phe	ttt Phe 620	gac Asp	atc Ile	aac Asn	atg Met	aaa Lys 625	ggt Gly	ttg Leu	gac Asp	ggt Gly	att Ile 630	caa Gln	2107
	gga Gly	ccc Pro	att Ile	tat Tyr 635	gtg Val	ggt Gly	act Thr	gga Gly	tgt Cys 640	gtt Val	ttc Phe	aga Arg	cgg Arg	cag Gln 645	gca Ala	ctg Leu	2155
	tat Tyr	ggt Gly	tat Tyr 650	gat Asp	gct Ala	cct Pro	aaa Lys	acg Thr 655	aag Lys	aag Lys	cca Pro	cca Pro	tca Ser 660	aga Arg	act Thr	tgc Cys	2203
	aac Asn	tgc Cys 665	tgg Trp	ccc Pro	aag Lys	tgg Trp	tgc Cys 670	ctc Leu	tct Ser	tgc Cys	tgc Cys	tgc Cys 675	agc Ser	agg Arg	aac Asn	aag Lys	2251
	aat Asn 680	aaa Lys	aag Lys	aag Lys	act Thr	aca Thr 685	aaa Lys	cca Pro	aag Lys	acg Thr	gag Glu 690	aag Lys	aag Lys	aaa Lys	aga Arg	tta Leu 695	2299
	ttt Phe	ttc Phe	aag Lys	aaa Lys	gca Ala 700	gaa Glu	aac Asn	cca Pro	tct Ser	cct Pro 705	gca Ala	tat Tyr	gct Ala	ttg Leu	ggt Gly 710	gaa Glu	2347
	att Ile	gat Asp	gaa Glu	ggt Gly 715	gct Ala	cca Pro	ggt Gly	gct Ala	gat Asp 720	atc Ile	gag Glu	aag Lys	gcc Ala	gga Gly 725	atc Ile	gta Val	2395
	aat Asn	caa Gln	cag Gln 730	aaa Lys	cta Leu	gag Glu	aag Lys	aaa Lys 735	ttt Phe	Gly aaa	cag Gln	tct Ser	tct Ser 740	gtt Val	ttt Phe	gtc Val	2443
	gca Ala	tca Ser 745	aca Thr	ctt Leu	ctt Leu	gag Glu	aac Asn 750	gga Gly	gly aaa	acc Thr	ctg Leu	aag Lys 755	agc Ser	gca Ala	agt Ser	cca Pro	2491
	gct Ala 760	tct Ser	ctt Leu	ctg Leu	aag Lys	gaa Glu 765	gct Ala	ata Ile	cat His	gtt Val	atc Ile 770	agc Ser	tgc Cys	Gly	tac Tyr	gaa Glu 775	2539
	gac Asp	aag Lys	acc Thr	gac Asp	tgg Trp 780	gga Gly	aaa Ly:s	gag Glu	att Ile	ggc Gly 785	tgg Trp	att Ile	tac Tyr	gga Gly	tcg Ser 790	atc Ile .	2587
•	aca Thr	gag Glu	gat Asp	atc Ile 795	ttg Leu	act Thr	gga Gly	ttt Phe	aag Lys 800	atg Met	cac His	tgc Cys	cat His	ggc Gly 805	tgg Trp	cgg Arg	2635
;	tct Ser	att Ile	tac Tyr 810	tgc Cys	atc Ile	ccg Pro	aag Lys	cgg Arg 815	cct Pro	gca Ala	ttc Phe	aaa Lys	ggt Gly 820	tct Ser	gcg Ala	cct Pro	2683
	ctg Leu	aac Asn 825	ctt Leu	tcc Ser	gac Asp	cgt Arg	ctt Leu 830	cac His	cag Gln	gtc Val	ctt Leu	ege Arg 835	tgg Trp	gcc Ala	ctt Leu	gl ^a aaa	2731
						ttc Phe											2779

- 52 -

840					845					850					855	
	ggc Gly															2827
	tat Tyr													_		2875
	atc Ile															2923
	gcc Ala 905															2971
	atc Ile															3019
agg Arg	aac Asn	gag Glu	cag Gln	ttc Phe 940	tgg Trp	gtc Val	atc Ile	gga Gly	ggc Gly 945	gtt Val	tcg Ser	gcg Ala	cat His	ctg Leu 950	Phe	3067
	gtg Val															3115
	acc Thr															3163
	acg Thr 985	Phe					Leu					Thr				3211
	ctg Leu)					Val					Ser					3259
	glà aaa				Trp					Gly	-				Ala	3307
	tgg Trp			Val		-		_	Phe		_		_	Val		3355
	cag Gln		Arg	_	_	_		Val		-			Ile	_	_	3403
	tcg Ser 106	Ile					Trp					Pro				3451

								_	JJ -						
aag Lys 108	Ser	aac Asr	ggo Gly	ecg	teto Lev 108	ı Lev	gag Glu	gag Glu	tgt Cys	ggc Gly 109	/ Let	, Asb	tgo Cys	: a	
ctg gtt gta	ctgt ttaa tatt	gtc gtc agt agc	tgtt catt tata	gttg ggag cagt aagg	ga a ca g ga t ac a	ittet gaga geac	ttgo gagg atto	t gt t gc c ag	agat ctgc	agaa tgct cagt	acc gtt gta	acat tgtt ttcc	gtc gag ctt	cacg taaa ttta	gatttt gcatct ttaaaa cagtct aaaaaa
	<		26 109 PRT												
			Zea		s										
		400>													
1				5					10					15	Glu
			20					25		,		Ala	30		
		35					40					Gly 45			
	50					55					60	Asn		-	
65					70					75		Arg		_	80
				85					90			Leu		95	
			100					105				Asp	110		
		115					120					Asp 125			
	130					135					140	Tyr Val		_	_
145					150					155		Val Gln			160
				165					170					175	
			180					185				Lys	190		
		195					200					Pro 205			
	210					215					220	Ser			
225					230					235		Arg			240
				245					250			Asp		255	
			260					265				Lys	270		
		275					280					Val 285			
	290					295					300	His			
305					310					315		Glu			320
Ala	Met	Ser	Trp	Ile	Leu	Asp	Gln	Phe	Pro	Lys	Trp	Leu	Pro	Ile	Glu

				325					330					335	
Arg	Glu	Thr	Tyr 340	Leu	Asp	Arg	Leu	Ser 345	Leu	Arg	Phe	Asp	Lys 350		Gly
Gln	Pro	Ser 355	Gln	Leu	Ala	Pro	Ile 360	Asp	Phe	Phe	Val	Ser 365		Val	Asp
	Thr 370	Lys	Glu	Pro	Pro	Leu 375	Val	Thr	Ala	Asn	Thr 380	Val	Leu	Ser	Ile
Leu 385	Ser	Val	Asp	Tyr	Pro 390	Val	Glu	Lys	Val	Ser 395	Cys	Tyr	Val	Ser	Asp 400
			Ala	405					410					415	
			Lys 420					425					430		
		435	Glu				440					445		_	_
	450		Ala			455					460				
465			Phe		470					475					480
			Glu	485					490		_			495	
			Val 500					505					510		_
		515	Gly				520					525			
	530		Arg			535					540				
G19 545	Ala	Met	Asn	Ala	Leu 550	Val	Arg	Val	Ser	Ala 555	Val	Leu	Ser	Asn	Ala 560
~ ~ .	_	-	_	_	_	_	_	_			-				
			Leu	565					570					575	_
Ala	Ile	Lys	Glu 580	565 Ala	Met	Cys	Phe	Met 585	570 Met	Asp	Pro	Leu	Val 590	575 Gly	Lys
Ala Lys	Ile Val	Lys Cys 595	Glu 580 Tyr	565 Ala Val	Met Gln	Cys Phe	Phe Pro 600	Met 585 Gln	570 Met Arg	Asp Phe	Pro Asp	Leu Gly 605	Val 590 Ile	575 Gly Asp	Lys Lys
Ala Lys Asn	Ile Val Asp 610	Lys Cys 595 Arg	Glu 580 Tyr Tyr	565 Ala Val Ala	Met Gln Asn	Cys Phe Arg 615	Phe Pro 600 Asn	Met 585 Gln Val	570 Met Arg Val	Asp Phe Phe	Pro Asp Phe 620	Leu Gly 605 Asp	Val 590 Ile Ile	575 Gly Asp Asn	Lys Lys Met
Ala Lys Asn Lys 625	Ile Val Asp 610 Gly	Lys Cys 595 Arg Leu	Glu 580 Tyr Tyr	565 Ala Val Ala Gly	Met Gln Asn Ile 630	Cys Phe Arg 615 Gln	Phe Pro 600 Asn Gly	Met 585 Gln Val Pro	570 Met Arg Val Ile	Asp Phe Phe Tyr 635	Pro Asp Phe 620 Val	Leu Gly 605 Asp Gly	Val 590 Ile Ile Thr	575 Gly Asp Asn Gly	Lys Lys Met Cys 640
Ala Lys Asn Lys 625 Val	Ile Val Asp 610 Gly Phe	Lys Cys 595 Arg Leu Arg	Glu 580 Tyr Tyr Asp	S65 Ala Val Ala Gly Gln 645	Met Gln Asn Ile 630 Ala	Cys Phe Arg 615 Gln Leu	Phe Pro 600 Asn Gly Tyr	Met 585 Gln Val Pro	570 Met Arg Val Ile Tyr 650	Asp Phe Phe Tyr 635 Asp	Pro Asp Phe 620 Val Ala	Leu Gly 605 Asp Gly Pro	Val 590 Ile Ile Thr	575 Gly Asp Asn Gly Thr 655	Lys Lys Met Cys 640 Lys
Ala Lys Asn Lys 625 Val	Ile Val Asp 610 Gly Phe	Lys Cys 595 Arg Leu Arg	Glu 580 Tyr Tyr Asp Arg Ser 660	S65 Ala Val Ala Gly Gln 645 Arg	Met Gln Asn Ile 630 Ala Thr	Cys Phe Arg 615 Gln Leu Cys	Phe Pro 600 Asn Gly Tyr	Met 585 Gln Val Pro Gly Cys 665	570 Met Arg Val Ile Tyr 650 Trp	Asp Phe Phe Tyr 635 Asp	Pro Asp Phe 620 Val Ala Lys	Leu Gly 605 Asp Gly Pro	Val 590 Ile Ile Thr Lys Cys 670	575 Gly Asp Asn Gly Thr 655 Leu	Lys Lys Met Cys 640 Lys
Ala Lys Asn Lys 625 Val Lys	Ile Val Asp 610 Gly Phe Pro Cys	Lys Cys 595 Arg Leu Arg Pro Cys 675	Glu 580 Tyr Tyr Asp Arg Ser 660 Ser	S65 Ala Val Ala Gly Gln 645 Arg	Met Gln Asn Ile 630 Ala Thr	Cys Phe Arg 615 Gln Leu Cys	Phe Pro 600 Asn Gly Tyr Asn Asn 680	Met 585 Gln Val Pro Gly Cys 665 Lys	570 Met Arg Val Ile Tyr 650 Trp Lys	Asp Phe Tyr 635 Asp Pro Lys	Pro Asp Phe 620 Val Ala Lys Thr	Leu Gly 605 Asp Gly Pro Trp Thr 685	Val 590 Ile Ile Thr Lys Cys 670 Lys	575 Gly Asp Asn Gly Thr 655 Leu	Lys Lys Met Cys 640 Lys Ser
Ala Lys Asn Lys 625 Val Lys Cys	Ile Val Asp 610 Gly Phe Pro Cys Glu 690	Lys Cys 595 Arg Leu Arg Pro Cys 675 Lys	Glu 580 Tyr Tyr Asp Arg Ser 660 Ser	S65 Ala Val Ala Gly Gln 645 Arg Arg	Met Gln Asn Ile 630 Ala Thr Asn Arg	Cys Phe Arg 615 Gln Leu Cys Lys Leu 695	Phe Pro 600 Asn Gly Tyr Asn 680 Phe	Met 585 Gln Val Pro Gly Cys 665 Lys	570 Met Arg Val Ile Tyr 650 Trp Lys	Asp Phe Tyr 635 Asp Pro Lys	Pro Asp Phe 620 Val Ala Lys Thr Ala 700	Leu Gly 605 Asp Gly Pro Trp Thr 685 Glu	Val 590 Ile Ile Thr Lys 670 Lys	575 Gly Asp Asn Gly Thr 655 Leu Pro	Lys Lys Met Cys 640 Lys Ser Lys
Ala Lys Asn Lys 625 Val Lys Cys Thr	Ile Val Asp 610 Gly Phe Pro Cys Glu 690 Ala	Lys Cys 595 Arg Leu Arg Pro Cys 675 Lys	Glu 580 Tyr Tyr Asp Arg Ser 660 Ser Lys	S65 Ala Val Ala Gly Gln 645 Arg Arg Lys	Met Gln Asn Ile 630 Ala Thr Asn Arg Gly 710	Cys Phe Arg 615 Gln Leu Cys Lys Leu 695 Glu	Phe Pro 600 Asn Gly Tyr Asn 680 Phe	Met 585 Gln Val Pro Gly Cys 665 Lys Phe	570 Met Arg Val Ile Tyr 650 Trp Lys Lys Glu	Asp Phe Tyr 635 Asp Pro Lys Lys Gly 715	Pro Asp Phe 620 Val Ala Lys Thr Ala 700 Ala	Leu Gly 605 Asp Gly Pro Trp Thr 685 Glu Pro	Val 590 Ile Ile Thr Lys 670 Lys Asn	Asp Asn Gly Thr 655 Leu Pro Pro	Lys Lys Met Cys 640 Lys Ser Lys Ser Asp 720
Ala Lys Asn Lys 625 Val Lys Cys Thr Pro 705 Ile	Ile Val Asp 610 Gly Phe Pro Cys Glu 690 Ala Glu	Lys Cys 595 Arg Leu Arg Pro Cys 675 Lys Tyr	Glu 580 Tyr Tyr Asp Arg Ser 660 Ser Lys Ala	S65 Ala Val Ala Gly Gln 645 Arg Lys Leu Gly 725	Met Gln Asn Ile 630 Ala Thr Asn Arg Gly 710 Ile	Cys Phe Arg 615 Gln Leu Cys Lys Leu 695 Glu Val	Phe Pro 600 Asn Gly Tyr Asn 680 Phe Ile Asn	Met 585 Gln Val Pro Gly Cys 665 Lys Phe Asp	570 Met Arg Val Ile Tyr 650 Trp Lys Glu Gln 730	Asp Phe Tyr 635 Asp Pro Lys Lys Gly 715 Lys	Pro Asp Phe 620 Val Ala Lys Thr Ala 700 Ala Leu	Leu Gly 605 Asp Gly Pro Trp Thr 685 Glu Pro Glu	Val 590 Ile Ile Thr Lys 670 Lys Asn Gly	S75 Gly Asp Asn Gly Thr 655 Leu Pro Pro Ala Lys 735	Lys Lys Met Cys 640 Lys Ser Lys Ser Asp 720 Phe
Ala Lys Asn Lys 625 Val Lys Cys Thr Pro 705 Ile	Ile Val Asp 610 Gly Phe Pro Cys Glu 690 Ala Glu Gln	Lys Cys 595 Arg Leu Arg Pro Cys 675 Lys Tyr Lys Ser	Glu 580 Tyr Tyr Asp Arg Ser 660 ser Lys Ala Ala Ser 740	S65 Ala Val Ala Gly Gln 645 Arg Arg Lys Leu Gly 725 Val	Met Gln Asn Ile 630 Ala Thr Asn Arg Gly 710 Ile Phe	Cys Phe Arg 615 Gln Leu Cys Lys Leu 695 Glu Val	Phe Pro 600 Asn Gly Tyr Asn 680 Phe Ile Asn Ala	Met 585 Gln Val Pro Gly Cys 665 Lys Phe Asp Gln Ser 745	570 Met Arg Val Ile Tyr 650 Trp Lys Glu Gln 730 Thr	Asp Phe Tyr 635 Asp Pro Lys Lys Gly 715 Lys	Pro Asp Phe 620 Val Ala Lys Thr Ala 700 Ala Leu Leu	Leu Gly 605 Asp Gly Pro Trp Thr 685 Glu Pro Glu Glu	Val 590 Ile Ile Thr Lys 670 Lys Asn Gly Lys	S75 Gly Asp Asn Gly Thr 655 Leu Pro Pro Ala Lys 735 Gly	Lys Lys Met Cys 640 Lys Ser Lys Ser Asp 720 Phe
Ala Lys Asn Lys 625 Val Lys Cys Thr Pro 705 Ile Gly Thr	Ile Val Asp 610 Gly Phe Pro Cys Glu 690 Ala Glu Gln Leu	Lys Cys 595 Arg Leu Arg Pro Cys 675 Lys Tyr Lys Ser Lys 755	Glu 580 Tyr Tyr Asp Arg Ser 660 Ser Lys Ala Ala Ser 740 Ser	S65 Ala Val Ala Gly Gln 645 Arg Arg Lys Leu Gly 725 Val	Met Gln Asn Ile 630 Ala Thr Asn Arg Gly 710 Ile Phe Ser	Cys Phe Arg 615 Gln Leu Cys Lys Leu 695 Glu Val Val Pro	Phe Pro 600 Asn Gly Tyr Asn 680 Phe Ile Asn Ala Ala 760	Met 585 Gln Val Pro Gly Cys 665 Lys Phe Asp Gln Ser 745 Ser	570 Met Arg Val Ile Tyr 650 Trp Lys Glu Gln 730 Thr	Asp Phe Tyr 635 Asp Pro Lys Gly 715 Lys Leu Leu	Pro Asp Phe 620 Val Ala Lys Thr Ala 700 Ala Leu Leu Lys	Leu Gly 605 Asp Gly Pro Trp Thr 685 Glu Pro Glu Glu Glu 765	Val 590 Ile Ile Thr Lys 670 Lys Asn Gly Lys Asn 750 Ala	S75 Gly Asp Asn Gly Thr 655 Leu Pro Pro Ala Lys 735 Gly Ile	Lys Lys Met Cys 640 Lys Ser Lys Ser Asp 720 Phe Gly His
Ala Lys Asn Lys 625 Val Lys Cys Thr Pro 705 Ile Gly Thr Val	Ile Val Asp 610 Gly Phe Pro Cys Glu 690 Ala Glu Gln Leu Ile 770	Lys Cys 595 Arg Leu Arg Pro Cys 675 Lys Tyr Lys Ser Lys 755 Ser	Glu 580 Tyr Tyr Asp Arg Ser 660 ser Lys Ala Ala Ser 740	S65 Ala Val Ala Gly Gln 645 Arg Lys Leu Gly 725 Val Ala Gly	Met Gln Asn Ile 630 Ala Thr Asn Arg Gly 710 Ile Phe Ser Tyr	Cys Phe Arg 615 Gln Leu Cys Lys Leu 695 Glu Val Val Pro Glu 775	Phe Pro 600 Asn Gly Tyr Asn 680 Phe Ile Asn Ala 760 Asp	Met 585 Gln Val Pro Gly Cys 665 Lys Phe Asp Gln Ser 745 Ser Lys	570 Met Arg Val Ile Tyr 650 Trp Lys Glu Gln 730 Thr Leu Thr	Asp Phe Tyr 635 Asp Pro Lys Gly 715 Lys Leu Leu Asp	Pro Asp Phe 620 Val Ala Lys Thr Ala 700 Ala Leu Leu Lys Trp 780	Leu Gly 605 Asp Gly Pro Trp Thr 685 Glu Pro Glu Glu Glu 765 Gly	Val 590 Ile Ile Thr Lys 670 Lys Asn Gly Lys Asn 750 Ala	S75 Gly Asp Asn Gly Thr 655 Leu Pro Pro Ala Lys 735 Gly Ile Glu	Lys Lys Met Cys 640 Lys Ser Lys Ser Gly His Ile

- 55 -

```
785
                  790
                                    795
Met His Cys His Gly Trp Arg Ser Ile Tyr Cys Ile Pro Lys Arg Pro
        805 810
Ala Phe Lys Gly Ser Ala Pro Leu Asn Leu Ser Asp Arg Leu His Gln
                            825
Val Leu Arg Trp Ala Leu Gly Ser Val Glu Ile Phe Phe Ser Lys His
                        840
Cys Pro Leu Trp Tyr Gly Tyr Gly Gly Leu Lys Phe Leu Glu Arg
                     855
                                       860
Phe Ser Tyr Ile Asn Ser Ile Val Tyr Pro Trp Thr Ser Ile Pro Leu
                 870
                                   875
Leu Ala Tyr Cys Thr Leu Pro Ala Ile Cys Leu Leu Thr Gly Lys Phe
             885
                               890
Ile Thr Pro Glu Leu Thr Asn Val Ala Ser Ile Trp Phe Met Ala Leu
          900 905
Phe Ile Cys Ile Ser Val Thr Gly Ile Leu Glu Met Arg Trp Ser Gly
                         920
                                           925
Val Ala Ile Asp Asp Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly
                     935
                                       940
Gly Val Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val
                 950
                                   955
Phe Ala Gly Ile Asp Thr Ser Phe Thr Val Thr Ser Lys Ala Gly Asp
              965
                               970
Asp Glu Glu Phe Ser Glu Leu Tyr Thr Phe Lys Trp Thr Thr Leu Leu
                             985
Ile Pro Pro Thr Thr Leu Leu Leu Leu Asn Phe Ile Gly Val Val Ala
                         1000
                                           1005
Gly Ile Ser Asn Ala Ile Asn Asn Gly Tyr Glu Ser Trp Gly Pro Leu
   1010
                     1015
                                       1020
Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro
                                   1035
                  1030
Phe Leu Lys Gly Leu Val Gly Arg Gln Asn Arg Thr Pro Thr Ile Val
                               1050
Ile Val Trp Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val
                           1065 1070
Arg Val Asp Pro Phe Leu Ala Lys Ser Asn Gly Pro Leu Leu Glu Glu
       1075
                       1080
Cys Gly Leu Asp Cys Asn
   1090
     <210> 27
     <211> 25
     <212> DNA
     <213> Zea mays
     <400> 27
atggaggcta gcgcggggct ggtgg
     <210> 28
     <211> 25
     <212> DNA
     <213> Zea mays
     <400> 28
tcagttgcag tccaggccac actcc
```

25

- 56 *-*

<210> 29 <211> 3746 <212> DNA <213> Zea mays <220> <221> CDS <222> (321)...(3549) <400> 29 ctaggatcaa aaccgtctcg ccgctgcaat aatcttttgt caattcttaa tccctcgcgt 60 cgacagcgac agcggaacca actcacgttg ccgcggcttc ctccatcggt gcggtgccct 120 180 actagcagca gcagcgctct cgcagcggga gatgcggtgt tgatccgtgc cccgctcgga tetegggaet ggtgeegget etgeeeagge eecaggetee aggeeagete cetegaegtt 300 teteggegag etegettgee atg gag gge gae geg gae gge gtg aag teg ggg 353 Met Glu Gly Asp Ala Asp Gly Val Lys Ser Gly agg cgc ggt ggc gga cag gtg tgc cag atc tgc ggc gac ggc gtg ggc 401 Arg Arg Gly Gly Gln Val Cys Gln Ile Cys Gly Asp Gly Val Gly 15 acc acg gcg gag ggg gac gtc ttc gcc gcc tgc gac gtc tgc ggg ttt 449 Thr Thr Ala Glu Gly Asp Val Phe Ala Ala Cys Asp Val Cys Gly Phe 30 ccg gtg tgc cgc ccc tgc tac gag tac gag cgc aag gac ggc acg cag 497 Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln 45 geg tgc ccc cag tgc aag acc aag tac aag cgc cac aag ggg agc ccg 545 Ala Cys Pro Gln Cys Lys Thr Lys Tyr Lys Arg His Lys Gly Ser Pro 60 65 geg ate egt ggg gag gaa gga gac gac act gat gee gat age gae tte 593 Ala Ile Arg Gly Glu Glu Gly Asp Asp Thr Asp Ala Asp Ser Asp Phe aat tac ctt gca tct ggc aat gag gac cag aag cag aag att gcc gac 641 Asn Tyr Leu Ala Ser Gly Asn Glu Asp Gln Lys Gln Lys Ile Ala Asp 100 aga atg cgc agc tgg cgc atg aac gtt ggg ggc agc ggg gat gtt ggt 689 Arg Met Arg Ser Trp Arg Met Asn Val Gly Gly Ser Gly Asp Val Gly cgc ccc aag tat gac agt ggc gag atc ggg ctt acc aag tat gac agt 737 Arg Pro Lys Tyr Asp Ser Gly Glu Ile Gly Leu Thr Lys Tyr Asp Ser 130 ggc gag att cet egg gga tac atc eca tea gtc act aac agc eag atc 785 Gly Glu Ile Pro Arg Gly Tyr Ile Pro Ser Val Thr Asn Ser Gln Ile 140 145 tca gga gaa atc cct ggt gct tcc cct gac cat cat atg atg tcc cca 833 Ser Gly Glu Ile Pro Gly Ala Ser Pro Asp His His Met Met Ser Pro 160 165 170

WO 00/09706

act Thr	ggg ggg	aac Asn	att Ile 175	ggc	aag Lys	cgt Arg	gct Ala	cca Pro 180	ttt Phe	ccc Pro	tat Tyr	gtg Val	aac Asn 185	cat His	tcg Ser	881
cca Pro	aat Asn	ccg Pro 190	tca Ser	agg Arg	gag Glu	ttc Phe	tct Ser 195	ggt Gly	agc Ser	att Ile	Gly 999	aat Asn 200	gtt Val	gcc Ala	tgg Trp	929
aaa Lys	gag Glu 205	agg Arg	gtt Val	gat Asp	ggc Gly	tgg Trp 210	aaa Lys	atg Met	aag Lys	cag Gln	gac Asp 215	aag Lys	ggg	acg Thr	att Ile	97 <i>7</i>
ccc Pro 220	atg Met	acg Thr	aat Asn	ggc Gly	aca Thr 225	agc Ser	att Ile	gct Ala	ccc Pro	tct Ser 230	gag Glu	ggt Gly	cgg Arg	ggt Gly	gtt Val 235	1025
ggt Gly	gat Asp	att Ile	gat Asp	gca Ala 240	tca Ser	act Thr	gat Asp	tac Tyr	aac Asn 245	atg Met	gaa Glu	gat Asp	gcc Ala	tta Leu 250	ttg Leu	1073
aac Asn	gac Asp	gaa Glu	act Thr 255	cga Arg	cag Gln	cct Pro	cta Leu	tct Ser 260	agg Arg	aaa Lys	gtt Val	cca Pro	ctt Leu 265	cct Pro	tcc Ser	1121
tcc Ser	agg Arg	ata Ile 270	aat Asn	cca Pro	tac Tyr	agg Arg	atg Met 275	gtc Val	att Ile	gtg Val	ctg Leu	cga Arg 280	ttg Leu	att Ile	gtt Val	1169
cta Leu	agc Ser 285	atc Ile	ttc Phe	ttg Leu	cac His	tac Tyr 290	cgt Arg	atc Ile	aca Thr	aat Asn	cct Pro 295	gtg Val	cgc Arg	aat Asn	gca Ala	1217
tac Tyr 300	cca Pro	tta Leu	tgg Trp	ctt Leu	cta Leu 305	tct Ser	gtt Val	ata Ile	tgt Cys	gag Glu 310	atc Ile	tgg Trp	ttt Phe	gct Ala	ctt Leu 315	1265
tcg Ser	tgg Trp	ata Ile	ttg Leu	gat Asp 320	cag Gln	ttc Phe	cct Pro	aag Lys	tgg Trp 325	ttt Phe	cca Pro	atc Ile	aac Asn	cgg Arg 330	gag Glu	1313
acg Thr	tac Tyr	Leu	gat Asp 335	agg Arg	ctg Leu	gca Ala	tta Leu	agg Arg 340	tat Tyr	gac Asp	cgg Arg	gaa Glu	ggt Gly 345	gag Glu	cca Pro	1361
tct Ser	cag Gln	ttg Leu 350	gct Ala	gct Ala	gtt Val	gac Asp	att Ile 355	ttc Phe	gtc Val	agt Ser	aca Thr	gtc Val 360	gac Asp	cca Pro	atg Met	1409
aag Lys	gag Glu 365	cct Pro	cct Pro	ctt Leu	gtc Val	act Thr 370	gcc Ala	aat Asn	acc Thr	gtg Val	cta Leu 375	tcc Ser	att Ile	ctt Leu	gct Ala	1457
	gat Asp															1505
gct	gcg	atg	ctg	aca	ttt	gat	gca	cta	gct	gag	act	tca	gag	ttt	gct	1553

- 58 -

Ala	Ala	Met	Leu	Thr 400	Phe	Asp	Ala	Leu	Ala 405	Glu	Thr	Ser	Glu	Phe 410	Ala	
	aaa															1601
Arg	Lys	Trp	Val 415	Pro	Phe	Val	Lys	Lys 420	Tyr	Asn	Ile	Glu		Arg	Ala	
			413					420					425			
	gaa															1649
Pro	Glu		Tyr	Phe	Ser	Gln		Ile	Asp	Tyr	Leu	_	Asp	Lys	Val	
		430					435					440				
	cct															1697
His	Pro 445	Ser	Phe	Val	Lys		Arg	Arg	Ala	Met		Arg	Glu	Tyr	Glu	
	445					450					455					
	ttc															1745
	Phe	Lys	Val	Arg		Asn	Gly	Leu	Val		Lys	Ala	Gln	Lys		
460					465					470					475	
	gag															1793
Pro	Glu	Glu	Gly		Ile	Met	Gln	Asp		Thr	Pro	Trp	Pro	-	Asn	
				480					485					490		
aat	acc	mgg	gac	cat	cct	gga	atg	att	cag	gtt	ttc	ctt	ggt	cac	agt	1841
Asn	Thr	Xaa		His	Pro	Gly	Met		Gln	Val	Phe	Leu	_	His	Ser	
			495					500					505			
															gtt.	1889
Gly	Gly		Asp	Thr	Glu	Gly		Glu	Leu	Pro	Arg		Val	Tyr	Val	
		510					515					520				
	cgt							_							-	1937
Ser	Arg	Glu	Lys	Arg	Pro		Phe	Gln	His	His	-	Lys	Ala	Gly	Ala	
	525					530					535					
	aat															1985
Met 540	Asn	Ala	Leu	Val	Arg 545	Val	Ser	Ala	Val		Thr	Asn	Gly	Gln	-	
340					343					550					555	
	ttg															2033
Met	Leu	Asn	Leu		Cys	Asp	His	Tyr		Asn	Asn	Ser	Lys		Leu	
				560					565					570		
	gaa															2081
Arg	Glu	Ala		Cys	Phe	Leu	Met	_	Pro	Asn	Leu	Gly	_	Ser	Val	
			575					580					585			
_	tac	-	-			_	_		-			_			-	2129
Cys	Tyr		Gln	Phe	Pro	Gln	_	Phe	Asp	Gly	Ile	-	Arg	Asn	Asp	
		590					595					600				
_	tat	_								_			_	_		2177
Arg	Tyr	Ala	Asn	Arg	Asn		Val	Phe	Phe	Asp		Asn	Leu	Arg	Gly	
	605					610					615					
ctt	gat	ggc	atc	caa	gga	cca	gtt	tat	gtc	gga	act	ggc	tgt	gtt	ttc	2225
	Asp	Gly	Ile	Gln	_	Pro	Val	Tyr	Val	_	Thr	Gly	Сув	Val		
620					625					630					635	

WO 00/09706

				640)	ggt	TYL	GI	64!	o Pr 5	0 Il	e Ly	s Gi	ln L	ys 50	Lys	2273
ggt Gly	ggt Gly	ttc Phe	ttg Leu 655	tca Ser	tca Ser	cta Leu	tgt Cys	G13 G13	GI	t ag y Ar	g aa g Ly	g aa s Ly	g go s Al	a se	gc :	aaa Lys	2321
tca Ser	-	aag Lys 670	ggc Gly	tcg Ser	gac Asp	aag Lys	aag Lys 675	aag Lys	tco Ser	g cag Gli	g aag n Lyg	g ca s Hi 68	s Va	g ga l As	ac a	agt Ser	2369
tct Ser	gtg Val 685	cca Pro	gta Val	ttc Phe	aac Asn	ctt Leu 690	gaa Glu	gat Asp	ata Ile	gag Glu	g gag ı Glı 695	ı Gly	agt YVa	t ga 1 Gl	ıa ç u G	gc Hy	2417
gct (Ala (700	gga 1 Gly 1	tt Phe	gac Asp	gac Asp	gag Glu 705	aaa Lys	tca Ser	ctt Leu	ctt Leu	atg Met 710	Ser	caa Glr	a atq	g ag t Se	r L	tg eu 15	2465
gag a	aag a Lys A	iga i		ggc Gly 720	cag Gln	tcc : Ser .	gca Ala	gcg Ala	ttt Phe 725	gtt Val	gcc	tcc Ser	act Thi	Ct Le	u M	tg et	2513
gag t Glu 1	at g Tyr G	-2 •	ggt 31y 735	gtt Val	cct Pro	cag i	ser.	gca Ala 740	act Thr	ccg Pro	gag Glu	tct Ser	ctt Leu 745	Lei	ga u L	aa ys	2561
gaa g Glu A		tc c le H 50	at q	gtt Val	ata : Ile :	oer (gt g Tys ('55	ggc Gly	tat Tyr	gag Glu	gac Asp	aag Lys 760	act Thr	gaa Glu	to 1 Tr	cb 33	2609
gga a Gly T 7	ct g hr G 65	ag a lu I	te d	gly '	11D .	atc t lle T 770	ac g yr (ggt Bly	tct Ser	gtg Val	aca Thr 775	gaa Glu	gac Asp	att	: ct : Le	:c :u	2657
acc g Thr G 780	ga ti ly Pl	c a ne L	ag a ys M		ac g His A 785	jeg e	ga g rg G	gc ly '	rp.	cgg Arg 790	tcg Ser	atc Ile	tac Tyr	tgc Cys	at Me 79	t	2705
ccc as	ag co	g P		ct t la F 00	tc a	ag g	gg t ly s	er A	gcc (Ala 1 305	ccc Pro	atc Ile	aat Asn	ctt Leu	tcg Ser 810	ga As	c p	2753
cgt ct Arg Le	g aa eu As	c ca n G]		tg c	tc c eu A	gg to rg Tı	p A	ct d la I 20	ett g Leu (317 : 333	tcc (Ser	Val (gag Glu 825	atc Ile	ct: Le:	c u	2801
ttc ag Phe Se	r eg r Ar 83	g	s C	As b Ac c	cc c ro L	tg tg eu Tr 83	р т	ac g yr G	gc t	ac (Tyr (Gly (999 (Gly 1	cgg Arg	ctc Leu	aaç Lys	J S	2849
ttc ct Phe Le 84		g ag u Ar	a tt g Pl	ic g	cg ta la Ty 89	/r 11	c aa e Aa	ac a sn T	cc a	hr 1	atc t [le]	tac o Tyr I	ccg Pro	ctc Leu	acg Thr	; :	2897
tcc at	c cc	gct	t ct	c at	c ta	tc tg	c at	c c	tg c	cc g	jec a	atc t	gt (ctg	ctc	:	2945

- 60 -

Ser Ile 860			665				870					875	
acc gga Thr Gly	aag t	c atc ne Ile 880	att c Ile P	ca gaq ro Gli	g atc ı Ile	agc Ser 885	Asn	ttc Phe	gcc Ala	agc Ser	atc Ile 890	tgg Trp	2993
ttc atc Phe Ile	tcc ct Ser Le	u Phe	atc t Ile S	eg ato	ttc Phe 900	Ala	acg Thr	ggc Gly	atc Ile	ctg Leu 905	gag Glu	atg Met	3041
agg tgg Arg Trp	agc gg Ser Gl 910	g gtg y Val	ggc a Gly I	c gac le Asp 915	Glu	tgg Trp	tgg Trp	agg Arg	aac Asn 920	gag Glu	cag Gln	ttc Phe	3089
tgg gtg Trp Val 925	atc gg Ile Gl	g ggc y Gly	atc to Ile Se 93	r Ala	cac His	ctc Leu	ttc Phe	gcc Ala 935	gtg Val	ttc Phe	cag Gln	ggc Gly	3137
ctg ctc Leu Leu 940	aag gt Lys Va	r neu .	gcc go Ala Gl 945	c atc y Ile	gac Asp	acc Thr	aac Asn 950	ttc Phe	acc Thr	gtc Val	Thr	tcc Ser 955	3185
aag gcc Lys Ala	tog ga Ser As	gag g Glu i 960	gac gg Asp Gl	c gac y Asp	ttc Phe	gcg Ala 965	gag Glu	ctg Leu	tàc : Tyr I	Met	ttc Phe : 970	aag Lys	3233
tgg acg a	acg cto Thr Let 97:	Leu.	atc cc Ile Pr	g ccc o Pro	acc Thr 980	acc Thr	atc (ctg : Leu :	Ile :	atc a [le 2 985	aac (Asn 1	ctg Leu	3281
gtc ggc g Val Gly V	gtc gtc Val Val 990	gee g	ggc at Sly Il	c tcc e Ser 995	Tyr :	gcc : Ala :	atc a Ile A	Asn S	agc g Ser G	ga t Sly T	tac (Tyr (eag Hn	3329
tcg tgg g Ser Trp 0 1005	gc ccg	ctc t Leu P	tc gg he Gl	Lys	ctc (Leu)	ttc (Phe 1	Phe A	jee t Ala F 1015	tc t	aa s	jtc a /al I	itc le	3377
gtc cac c Val His I 1020	cu lyr	ccg t Pro P	ne rei	aag Lys	ggc (Leu N	atg g Met G LO30	gc a	igg c	ag a ln A	sn A	gc rg 035	3425
acc ccg a Thr Pro T	cc atc hr Ile	gtc g Val V 1040	tc gtc al Val	tgg Trp	Ala I	atc c le L 1045	tg c eu L	tg g eu A	cg t la S	er I	tc t le P 050	tc he	3473
tcc ttg c Ser Leu L	tg tgg eu Trp 105	Val A	gc ato rg Ile	Asp :	ccc t Pro P 1060	tc a	icc a hr T	cc c hr A	rg Va	tc a al T	ct g hr G	gc ly	3521
ccg gat a Pro Asp Ti	cc cag hr Gln 070	acg to	gt ggc /s Gly	atc a Ile 1 1075	aac t Asn	gct	aggga	aag 1	tggaa	aggt	tt		3569
gtactttgta ataagcagca aagttttact	a agugg	cgtta	tttac	aqcta	cota	Caga	cc ac	**~~	a + a + +	- ~			3629 3689 3746

<210> 30 <211> 1077 <212> PRT <213> Zea mays

<213> Zea mays <400> 30 Met Glu Gly Asp Ala Asp Gly Val Lys Ser Gly Arg Arg Gly Gly Gly 10 Gln Val Cys Gln Ile Cys Gly Asp Gly Val Gly Thr Thr Ala Glu Gly 25 Asp Val Phe Ala Ala Cys Asp Val Cys Gly Phe Pro Val Cys Arg Pro 40 Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln Ala Cys Pro Gln Cys 55 Lys Thr Lys Tyr Lys Arg His Lys Gly Ser Pro Ala Ile Arg Gly Glu 70 Glu Gly Asp Asp Thr Asp Ala Asp Ser Asp Phe Asn Tyr Leu Ala Ser 90 Gly Asn Glu Asp Gln Lys Gln Lys Ile Ala Asp Arg Met Arg Ser Trp 105 Arg Met Asn Val Gly Gly Ser Gly Asp Val Gly Arg Pro Lys Tyr Asp 120 125 Ser Gly Glu Ile Gly Leu Thr Lys Tyr Asp Ser Gly Glu Ile Pro Arg 135 140 Gly Tyr Ile Pro Ser Val Thr Asn Ser Gln Ile Ser Gly Glu Ile Pro 150 155 Gly Ala Ser Pro Asp His His Met Met Ser Pro Thr Gly Asn Ile Gly 170 Lys Arg Ala Pro Phe Pro Tyr Val Asn His Ser Pro Asn Pro Ser Arg 185 Glu Phe Ser Gly Ser Ile Gly Asn Val Ala Trp Lys Glu Arg Val Asp 200 205 Gly Trp Lys Met Lys Gln Asp Lys Gly Thr Ile Pro Met Thr Asn Gly 215 220 Thr Ser Ile Ala Pro Ser Glu Gly Arg Gly Val Gly Asp Ile Asp Ala 230 235 Ser Thr Asp Tyr Asn Met Glu Asp Ala Leu Leu Asn Asp Glu Thr Arg 250 Gln Pro Leu Ser Arg Lys Val Pro Leu Pro Ser Ser Arg Ile Asn Pro 260 265 Tyr Arg Met Val Ile Val Leu Arg Leu Ile Val Leu Ser Ile Phe Leu 280 His Tyr Arg Ile Thr Asn Pro Val Arg Asn Ala Tyr Pro Leu Trp Leu 295 300 Leu Ser Val Ile Cys Glu Ile Trp Phe Ala Leu Ser Trp Ile Leu Asp 310 315 Gln Phe Pro Lys Trp Phe Pro Ile Asn Arg Glu Thr Tyr Leu Asp Arg 330 Leu Ala Leu Arg Tyr Asp Arg Glu Gly Glu Pro Ser Gln Leu Ala Ala 345 350 Val Asp Ile Phe Val Ser Thr Val Asp Pro Met Lys Glu Pro Pro Leu 360 Val Thr Ala Asn Thr Val Leu Ser Ile Leu Ala Val Asp Tyr Pro Val 375 380 Asp Lys Val Ser Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr 390 395

Phe Asp Ala Leu Ala Glu Thr Ser Glu Phe Ala Arg Lys Trp Val Pro

										02						
рh	a 17:	. 1	.		40	5				4]	LO				41	L 5
					·				42	5						r Phe
								441)				4 4	o Se	er Ph	e Val
							45	`					u Ph	e Ly		l Arg
	_					49/(,				47	l Pr	o Gl			y Trp
Il	e Me	t (3ln	Asp	Gl:	y Thi	Pro	Trp	Pro	G1 49	y As	n As	n Th	r Xa		480 p His
Pr	o Gl	уМ	let	11e	e Gli	n Val	Phe	Leu	Gly 509	/ Hi	s Se	r Gl	y Gl			5 p Thr
Glı	ı Gl	y F	Asn 515	Glu	ı Let	ı Pro	Arg	Leu 520	Va]	L Ty	r Va	l Se	r Arg	51 g Gl	o u Ly	s Arg
		-					235	Lys	Ala			EAL	^	n Al		u Val
Arg 545	y Va S	1 s	er	Ala	Val	Leu 550	Thr	Asn	Gly	Gl:	1 Ty:	r Mei	t Lei	ı Ası	n Lei	u Asp
					202	,				576	a Lei	u Arg				560 Cys
Phe	Lei	u M	et	Asp 580	Pro	Asn	Leu	Gly	Arg 585	Sea	· Va:	l Cys	з Туг			Phe
		_						h()()	Arg	Asr			~ ^ ~ =		Asr	Arg
		-					0 7 2	Asn				/ Leu	Asp	Gl		Gln
						030					676	Asn	Arg			Leu
					0.40	Pro				650	Lys	Gly				
				000		Arg			665						Gly	Ser
		•	, _			Gln		680					C 0 =	Pro	Val	
						Glu	כעם					700	Gly			
						Met 710					715	Glu				700
					143	Val				720						Val
			•			Pro			145					760	His	
		, ,	_			Glu .		760					765			
						Val	//5					780				
						Arg : 790					795					000
					805	Pro :				ยาก	Asp				016	
Leu	Arg	Tr	A 9 8	la 1 20	Leu (Gly s	Ser (/al (31u :	Ile	Leu	Phe			815 His	Cys
Pro	Leu	Tr ₁ 839	Т		3ly '	Tyr (Sly G	31y #	arg	Leu	Lys		Leu 845	830 Glu	Arg	Phe
	0.50					8	[le T	yr E				Ser	Ile			
Ile '	Tyr	Cys	3 I.	le I	Leu 1	Pro A	Ala I	le C	ys I	Leu	Leu	Thr	Gly :	Lys	Phe	Ile

- 63 -

890

Ile Pro Glu Ile Ser Asn Phe Ala Ser Ile Trp Phe Ile Ser Leu Phe

Ile Ser Ile Phe Ala Thr Gly Ile Leu Glu Met Arg Trp Ser Gly Val

875

870

885

865

```
905
  Gly Ile Asp Glu Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly
                             920
                                                 925
  Ile Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu
                         935
                                             940
  Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu
                     950
                                        955
 Asp Gly Asp Phe Ala Glu Leu Tyr Met Phe Lys Trp Thr Thr Leu Leu
                                     970
 Ile Pro Pro Thr Thr Ile Leu Ile Ile Asn Leu Val Gly Val Val Ala
             980
                               985
 Gly Ile Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu
                            1000
                                                 1005
 Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro
                        1015
                                            1020
 Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val
 1025
                    1030
                                        1035
 Val Val Trp Ala Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val
                1045
                                     1050
 Arg Ile Asp Pro Phe Thr Thr Arg Val Thr Gly Pro Asp Thr Gln Thr
            1060
                                1065
 Cys Gly Ile Asn Cys
         1075
       <210> 31
       <211> 25
       <212> DNA
       <213> Zea mays
       <400> 31
atggagggg acgcggacgg cgtga
       <210> 32
       <211> 25
       <212> DNA
       <213> Zea mays
      <400> 32
ctagcagttg atgccacacg tctgg
25
      <210> 33
      <211> 3753
      <212> DNA
      <213> Zea mays
      <220>
      <221> CDS
      <222> (184)...(3406)
      <400> 33
cagcagcaga agcactgcgc ggcattgcag cgatcgagcg ggaggaattt ggggcatggt
                                                                     60
ggtcgccaac gccgctcgga tctagaggcc cgcacgggcc gattggtctc cgcccgcctc
```

WO 00/09706

gtcggtgtt	g gtgtcgt	tgg cgtg	tggagc c	qtctcqqt	g ggaggad	icaa aas	Tanaaca	100
gag atg g	cg gcc aa la Ala As	c aag gg	g atg gt	g aca aa	c tcg cac y Ser His	aac co	220	180 228
gag ttc g Glu Phe V	tc atg at al Met Il 2	e Arg His	gac gg Asp Gl	c gat gt y Asp Vai 25	g ccg ggc l Pro Gly	tcg gct Ser Ala 30	a Lys	276
ccc aca a Pro Thr L	ag agt gc ys Ser Al	g aat gga a Asn Gl _l	a cag gto Gln Vai	l Cys Glı	g att tgo n Ile Cys	ggt gad Gly Asr 45	c tct Ser	324
gtg ggt g Val Gly Va	tt tca gca al Ser Ala 50	c act ggt a Thr Gly	gat gto Asp Val	ttt gtt l Phe Val	gcc tgc L Ala Cys 60	Asn Glu	tgt Cys	372
gcc ttc cc Ala Phe Pi 65	et gtc tgo co Val Cys	c cgc cca s Arg Pro 70	Cys Tyr	gag tat Glu Tyr	gag cgc Glu Arg 75	aag gag Lys Glu	gly aaa	420
aac caa to Asn Gln Cy 80	gc tgc cco /s Cys Pro	cag tgc Gln Cys 85	aag act	aga tac Arg Tyr	Lys Arg	cag aaa Gln Lys	ggt Gly 95	468
agc cct co Ser Pro Ar	ga gtt cat g Val His 100	GIY Asp	gag gat Glu Asp	gag gaa Glu Glu 105	gat gtt Asp Val	gat gac Asp Asp 110	Leu	516
gac aat ga Asp Asn Gl	a ttc aac u Phe Asn 115	tac aag Tyr Lys	caa ggc Gln Gly 120	Ser Gly	aaa ggc	cca gag Pro Glu 125	tgg Trp	564
caa ctg ca Gln Leu Gl 13	n GIY Asp	gat gct Asp Ala	gat ctg Asp Leu 135	tct tca Ser Ser	tct gct Ser Ala 140	cgc cat Arg His	gag Glu	612
cca cat ca Pro His Hi 145	t cgg att s Arg Ile	cca cgc Pro Arg 150	ctg aca Leu Thr	agc ggt Ser Gly	caa cag Gln Gln 155	ata tct Ile Ser	gga Gly	660
gag att cc Glu Ile Pr 160	t gat gct o Asp Ala	tcc cct Ser Pro 165	gac cgt Asp Arg	cat tct His Ser 170	atc cgc Ile Arg	agt cca Ser Pro	aca Thr 175	708
tcg agc ta Ser Ser Ty:	t gtt gat r Val Asp 180	cca agc Pro Ser	gtc cca Val Pro	gtt cct Val Pro 185	gtg agg Val Arg	att gtg Ile Val 190	gac Asp	756
ccc tcg aag Pro Ser Lys	g gac ttg s Asp Leu 195	aat tcc Asn Ser	tat ggg Tyr Gly 200	ctt aat Leu Asn	Ser Val	gac tgg Asp Trp 205	aag Lys	804
gaa aga gtt Glu Arg Val 210	l Glu Ser	tgg agg Trp Arg	gtt aaa Val Lys 215	cag gac Gln Asp	aaa aat Lys Asn 220	atg atg Met Met	caa Gln	852
gtg act aat	aaa tat	cca gag	gct aga	gga gga	gac atg	gag ggg	act	900

- 65 -

Va:	L Th: 22!	c Ası 5	l Lys	з Туз	Pro	230	Ala	Arg	g Gly	/ Gly	/ As _I		: Gl	ı Gl	/ Thr	
gg(Gl) 240	/ Sei	a aat Asr	gga Gly	a gaa / Glu	nat Xaa 245	Met	caa Gln	atg Met	gtt Val	gat Asp 250	Ası	gca Ala	cgg Arg	g cta g Lei	cct Pro 255	948
t t <u>s</u>	g ago	c cgt : Arg	ato Ile	gtg Val 260	Pro	att Ile	tcc Ser	tca Ser	aac Asn 265	Gln	cto Lei	aac a Asn	ctt Leu	tac Tyr 270	cgg Arg	996
gta Val	gtg Val	ato Ile	att Ile 275	Leu	cgt Arg	ctt Leu	atc Ile	Ile 280	Leu	tgc Cys	ttc Phe	tto Phe	Phe 285	Gln	tat Tyr	1044
Arg	gto Val	ser Ser 290	Hls	cca Pro	gtg Val	cgt Arg	gat Asp 295	gct Ala	tat Tyr	gga Gly	tta Leu	tgg Trp 300	cta Leu	gta Val	tct	1092
gtt Val	Ile 305	Cys	gag Glu	gtc Val	tgg Trp	ttt Phe 310	gcc Ala	ttg Leu	tct Ser	tgg Trp	ctt Leu 315	Leu	gat Asp	cag Gln	ttc Phe	1140
cca Pro 320	Lys	tgg Trp	tat Tyr	cca Pro	atc Ile 325	aac	cgt Arg	gag Glu	aca Thr	tat Tyr 330	ctt Leu	gac Asp	agg Arg	ctt Leu	gca Ala 335	1188
Leu	Arg	Tyr	Asp	Arg 340	Glu	Gly	Glu	Pro	Ser 345	Gln	Leu	gct Ala	Pro	Ile 350	Asp	1236
gtc Val	ttc Phe	gtc Val	agt Ser 355	aca Thr	gtg Val	gat Asp	cca Pro	ttg Leu 360	aag Lys	gaa Glu	cct Pro	cca Pro	ctg Leu 365	atc Ile	aca Thr	1284
gcc Ala	aac Asn	act Thr 370	gtt Val	ttg Leu	tcc Ser	att Ile	ctt Leu 375	tct Ser	gtg Val	gat Asp	tac Tyr	cct Pro 380	gtt Val	gac Asp	aaa Lys	1332
gtg Val	tca Ser 385	tgc Cys	tat Tyr	gtt Val	tct Ser	gat Asp 390	gat Asp	ggt Gly	tca Ser	gct Ala	atg Met 395	ctg Leu	act Thr	ttt Phe	gag Glu	1380
tct Ser 400	ctc Leu	tca Ser	gaa Glu	acc Thr	gca Ala 405	gaa Glu	ttt Phe	gct Ala	Arg	aag Lys 410	tgg Trp	gtt Val	ccc Pro	ttt Phe	tgt Cys 415	1428
aag Lys	aag Lys	cac His	Asn	att Ile 420	gaa Glu	cca Pro	aga Arg	Ala	cca Pro 425	gaa Glu	ttt Phe	tac Tyr	ttt Phe	gct Ala 430	caa Gln	1476
aaa Lys	ata Ile	gat Asp	tac Tyr 435	ctg Leu	aag Lys .	gac Asp	Lys	att Ile 440	caa Gln	cct Pro	tca Ser	ttt Phe	gtt Val 445	aag Lys	gaa Glu	1524
aga Arg	Arg	gca Ala 450	atg Met	aag Lys	agg (Arg (Glu	tat Tyr 455	gaa Glu	gaa Glu	ttc Phe	aaa Lys	gta Val 460	aga Arg	atc Ile	aat Asn	1572

gcc	Leu 465	gtt Val	gcc Ala	aaa Lys	gca Ala	cag Gln 470	aaa Lys	gtg Val	cct Pro	gaa Glu	gag Glu 475	ggg Gly	tgg Trp	acc Thr	atg Met	1620
gct Ala 480	gat Asp	gga Gly	act Thr	gca Ala	tgg Trp 485	cct Pro	Gly 999	aat Asn	aat Asn	cct Pro 490	agg Arg	gac Asp	cat His	cct Pro	ggc Gly 495	1668
atg Met	att Ile	cag Gln	gtt Val	ttc Phe 500	ttg Leu	Gly aaa	cac His	agt Ser	ggt Gly 505	Gly 999	ctc Leu	gac Asp	act Thr	gat Asp 510	gga Gly	1716
aạt Asn	gag Glu	tta Leu	cca Pro 515	cgt Arg	ctt Leu	gtc Val	tat Tyr	gtc Val 520	tct Ser	cgt Arg	gaa Glu	aag Lys	aga Arg 525	cca Pro	ggc Gly	1764
ttt Phe	cag Gln	cat His 530	cac His	aag Lys	aag Lys	gct Ala	ggt Gly 535	gca Ala	atg Met	aat Asn	gcg Ala	ctg Leu 540	att Ile	cgt Arg	gta Val	1812
tct Ser	gct Ala 545	gtg Val	ctg Leu	aca Thr	aat Asn	ggt Gly 550	gcc Ala	tat Tyr	ctt Leu	ctc Leu	aat Asn 555	gtg Val	gat Asp	tgc Cys	gac Asp	1860
cat His 560	tac Tyr	ttc Phe	aat Asn	agc Ser	agc Ser 565	aaa Lys	gct Ala	ctt Leu	aga Arg	gaa Glu 570	gca Ala	atg Met	tgc Cys	ttc Phe	atg Met 575	1908
	gat Asp															1956
aga Arg	ttt Phe	gat Asp	ggc Gly 595	att Ile	gac Asp	ttg Leu	cac His	gat Asp 600	cga Arg	tat Tyr	gct Ala	aat Asn	cgg Arg 605	aac Asn	ata Ile	2004
gtt Val	ttc Phe	ttt Phe 610	gat Asp	atc Ile	aac Asn	atg Met	aaa Lys 615	ggt Gly	ctg Leu	gat Asp	ggc Gly	att Ile 620	cag Gln	ggt Gly	cca Pro	2052
gtt Val	tac Tyr 625	gtg Val	gga Gly	aca Thr	gga Gly	tgc Cys 630	tgt Cys	ttc Phe	aat Asn	aga Arg	cag Gln 635	gct Ala	ttg Leu	tat Tyr	gga Gly	2100
tac Tyr 640	gat Asp	cct Pro	gtt Val	ttg Leu	act Thr 645	gaa Glu	gct Ala	gat Asp	ctg Leu	gag Glu 650	cca Pro	aac Asn	att Ile	gtt Val	att Ile 655	2148
	agc Ser															2196
	caa Gln															2244
aat	atg	gaa	gac	atc	gaa	gag	ggt	att	gaa	ggt	tac	gag	gat	gaa	agg	2292

Asn	Met	Glu 690	Asp	Ile	Glu	Glu	Gly 695		Glu	Gly	тут	Glu 700	_	Glu	Arg	
tca Ser	gtg Val 705	ctt Leu	atg Met	tcc Ser	cag Gln	agg Arg 710	aaa Lys	ttg Leu	gag Glu	aaa Lys	cgc Arg 715	ttt Phe	ggt Gly	cag Gln	tct Ser	2340
cct Pro 720	att Ile	ttc Phe	att Ile	gca Ala	tcc Ser 725	acc Thr	ttt Phe	atg Met	aca Thr	caa Gln 730	ggt Gly	ggc Gly	ata Ile	cca Pro	cct Pro 735	2388
tca Ser	aca Thr	aac Asn	cca Pro	gct Ala 740	tct Ser	cta Leu	cta Leu	aag Lys	gaa Glu 745	gct Ala	atc Ile	cat His	gtc Val	atc Ile 750	agt Ser	2436
tgt Cys	gga Gly	tat Tyr	gag Glu 755	gac Asp	aaa Lys	act Thr	gaa Glu	tgg Trp 760	gga Gly	aaa Lys	gag Glu	att Ile	ggc Gly 765	Trp	atc Ile	2484
tat Tyr	ggt Gly	tca Ser 770	gta Val	acg Thr	gag Glu	gat Asp	att Ile 775	ctg Leu	act Thr	ggg Gly	ttt Phe	aaa Lys 780	atg Met	cat His	gca Ala	2532
agg Arg	ggc Gly 785	tgg Trp	caa Gln	tca Ser	atc Ile	tac Tyr 790	tgc Cys	atg Met	cca Pro	cca Pro	cga Arg 795	cct Pro	tgt Cys	ttc Phe	aag Lys	2580
ggt Gly 800	tct Ser	gca Ala	cca Pro	atc Ile	aat Asn 805	ctt Leu	tcc Ser	gat Asp	cgt Arg	ctt Leu 810	aat Asn	cag Gln	gtg Val	ctc Leu	cgt Arg 815	2628
tgg Trp	gct Ala	ctt Leu	Gly 999	tca Ser 820	gtg Val	gaa Glu	att Ile	ctg Leu	ctt Leu 825	agt Ser	aga Arg	cat His	tgt Cys	cct Pro 830	atc Ile	2676
tgg Trp	tat Tyr	ggt Gly	tac Tyr 835	aat Asn	gga Gly	cga Arg	ttg Leu	aag Lys 840	ctt Leu	ttg Leu	gag Glu	agg Arg	ctg Leu 845	gct Ala	tac Tyr	2724
atc Ile	aac Asn	act Thr 850	att Ile	gta Val	tat Tyr	cca Pro	atc Ile 855	aca Thr	tcc Ser	att Ile	ccg Pro	ctt Leu 860	att Ile	gcc Ala	tat Tyr	2772
tgt Cys	gtg Val 865	ctt Leu	ccc Pro	gct Ala	atc Ile	tgc Cys 870	ctc Leu	ctt Leu	acc Thr	aat Asn	aaa Lys 875	ttt Phe	atc Ile	att Ile	cct Pro	2820
gag Glu 880	att Ile	agc Ser	aat Asn	tat Tyr	gct Ala 885	gjy aaa	atg Met	ttc Phe	ttc Phe	att Ile 890	ctt Leu	ctt Leu	ttc Phe	gcc Ala	tcc Ser 895	2868
att Ile	ttt Phe	gcc Ala	act Thr	ggt Gly 900	ata Ile	ttg Leu	gag Glu	ctt Leu	aga Arg 905	tgg Trp	agt Ser	ggt Gly	gtt Val	ggc 910	att Ile	2916
gaa Glu	gat Asp	tgg Trp	tgg Trp 915	aga Arg	aat Asn	gag Glu	cag Gln	ttt Phe 920	tgg Trp	gtt Val	att Ile	ggt Gly	ggc Gly 925	acc Thr	tct Ser	2964

geo	cat His	ctc Leu 930	Phe	gca Ala	gtg Val	ttc Phe	cag Gln 935	ggt Gly	ctg Leu	ctg Leu	aaa Lys	gtg Val 940	ttg Leu	gct Ala	ggg	3012
att Ile	gat Asp 945	acc Thr	aac Asn	ttc Phe	aca Thr	gtt Val 950	acc Thr	tca Ser	aag Lys	gca Ala	tct Ser 955	gat Asp	gag Glu	gat Asp	ggc	3060
gac Asp 960	ttt Phe	gct Ala	gag Glu	cta Leu	tat Tyr 965	gtg Val	ttc Phe	aag Lys	tgg Trp	acc Thr 970	agt Ser	ttg Leu	ctc Leu	att Ile	cct Pro 975	3108
ccg Pro	acc Thr	act Thr	gtt Val	ctt Leu 980	gtc Val	att Ile	aac Asn	ctg Leu	gtc Val 985	gga Gly	atg Met	gtg Val	gca Ala	gga Gly 990	att Ile	3156
tct Ser	tat Tyr	gcc Ala	att Ile 995	Asn	agt Ser	ggc Gly	tac Tyr	caa Gln 1000	Ser	tgg Trp	ggt Gly	ccg Pro	ctc Leu 1009	Phe	gga Gly	3204
Lys	ctg Leu	Pne 1010	Phe	Ser	Ile	Trp	Val 1015	Ile	Leu	His	Leu	Tyr 1020	Pro	Phe	Leu	3252
Lys	ggt Gly 1025	Leu	Met	Gly	Arg	Gln 1030	Asn	Arg	Thr	Pro	Thr 1035	Ile	Val	Ile	Val	3300
1040		IIe	Leu	Leu	Ala 1045	Ser	Ile	Phe	Ser	Leu 1050	Leu	Trp	Val	Lys	Ile 1055	3348
gat Asp	cct Pro	ttc Phe	Ile .	tcc Ser 1060	Pro '	aca (cag . Gln :	Lys	gct Ala 1065	Ala .	gcc Ala :	ttg (Leu (Gly	caa Gln 1070	Cys	3396
ggc	gtc : Val :	aac Asn	t gc	tgat	cgag	acag	gtga	ctc	ttat	ttga	ag ag	ggct	caat	С		3446
gtga tgct aaga	ggate gegga tgtga tttgi	gg at ac ta aa tt	tttg: aagaa tttg:	atci itca iagti	t aag c gga t ttg	gttat agcct gttat	gcc ttc gcg	tace tace	gttc: cttc: agtt!	att a cat g cat t	agctt gtago cqttt	ctto gcca taga	ec gr ag co ag ta	tgcc; cagc;	tgtag ggtgc agcgt tatca aaaaa	3506 3566 3626 3686 3746 3753
	<21	l0> 3 l1> 1 l2> E	1075													

<213> Zea mays

<400> 34

Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu 1 10 15 Phe Val Met Ile Arg His Asp Gly Asp Val Pro Gly Ser Ala Lys Pro 25

Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Ser Val 40 Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala Phe Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Glu Gly Asn 70 75 Gln Cys Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Gln Lys Gly Ser 90 Pro Arg Val His Gly Asp Glu Asp Glu Glu Asp Val Asp Asp Leu Asp 105 Asn Glu Phe Asn Tyr Lys Gln Gly Ser Gly Lys Gly Pro Glu Trp Gln 120 125 Leu Gln Gly Asp Asp Ala Asp Leu Ser Ser Ser Ala Arg His Glu Pro 135 His His Arg Ile Pro Arg Leu Thr Ser Gly Gln Gln Ile Ser Gly Glu 150 155 Ile Pro Asp Ala Ser Pro Asp Arg His Ser Ile Arg Ser Pro Thr Ser 170 Ser Tyr Val Asp Pro Ser Val Pro Val Pro Val Arg Ile Val Asp Pro 185 Ser Lys Asp Leu Asn Ser Tyr Gly Leu Asn Ser Val Asp Trp Lys Glu 200 Arg Val Glu Ser Trp Arg Val Lys Gln Asp Lys Asn Met Met Gln Val 215 Thr Asn Lys Tyr Pro Glu Ala Arg Gly Gly Asp Met Glu Gly Thr Gly 230 235 Ser Asn Gly Glu Xaa Met Gln Met Val Asp Asp Ala Arg Leu Pro Leu 250 Ser Arg Ile Val Pro Ile Ser Ser Asn Gln Leu Asn Leu Tyr Arg Val 265 Val Ile Ile Leu Arg Leu Ile Ile Leu Cys Phe Phe Phe Gln Tyr Arg 280 Val Ser His Pro Val Arg Asp Ala Tyr Gly Leu Trp Leu Val Ser Val 295 Ile Cys Glu Val Trp Phe Ala Leu Ser Trp Leu Leu Asp Gln Phe Pro 310 , 315 Lys Trp Tyr Pro Ile Asn Arg Glu Thr Tyr Leu Asp Arg Leu Ala Leu 325 330 Arg Tyr Asp Arg Glu Gly Glu Pro Ser Gln Leu Ala Pro Ile Asp Val 340 345 Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro Leu Ile Thr Ala 360 Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro Val Asp Lys Val 375 Ser Cys Tyr Val Ser Asp Gly Ser Ala Met Leu Thr Phe Glu Ser 390 395 Leu Ser Glu Thr Ala Glu Phe Ala Arg Lys Trp Val Pro Phe Cys Lys 405 410 Lys His Asn Ile Glu Pro Arg Ala Pro Glu Phe Tyr Phe Ala Gln Lys 420 425 Ile Asp Tyr Leu Lys Asp Lys Ile Gln Pro Ser Phe Val Lys Glu Arg 440 Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala 455 460 Leu Val Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr Met Ala 470 475 Asp Gly Thr Ala Trp Pro Gly Asn Asn Pro Arg Asp His Pro Gly Met 485 490

- 70 -

Ile Gln Val Phe Leu Gly His Ser Gly Gly Leu Asp Thr Asp Gly Asn 500 505 Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro Gly Phe 520 Gln His His Lys Lys Ala Gly Ala Met Asn Ala Leu Ile Arg Val Ser 535 Ala Val Leu Thr Asn Gly Ala Tyr Leu Leu Asn Val Asp Cys Asp His 550 555 Tyr Phe Asn Ser Ser Lys Ala Leu Arg Glu Ala Met Cys Phe Met Met 565 570 Asp Pro Ala Leu Gly Arg Lys Thr Cys Tyr Val Gln Phe Pro Gln Arg 580 585 Phe Asp Gly Ile Asp Leu His Asp Arg Tyr Ala Asn Arg Asn Ile Val 600 Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly Pro Val 615 Tyr Val Gly Thr Gly Cys Cys Phe Asn Arg Gln Ala Leu Tyr Gly Tyr 630 635 Asp Pro Val Leu Thr Glu Ala Asp Leu Glu Pro Asn Ile Val Ile Lys 645 650 Ser Cys Cys Gly Arg Arg Lys Lys Lys Asn Lys Ser Tyr Met Asp Ser 660 665 Gln Ser Arg Ile Met Lys Arg Thr Glu Ser Ser Ala Pro Ile Phe Asn 680 Met Glu Asp Ile Glu Glu Gly Ile Glu Gly Tyr Glu Asp Glu Arg Ser 695 Val Leu Met Ser Gln Arg Lys Leu Glu Lys Arg Phe Gly Gln Ser Pro 710 715 Ile Phe Ile Ala Ser Thr Phe Met Thr Gln Gly Gly Ile Pro Pro Ser 730 Thr Asn Pro Ala Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys 745 Gly Tyr Glu Asp Lys Thr Glu Trp Gly Lys Glu Ile Gly Trp Ile Tyr 760 Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Ala Arg 775 Gly Trp Gln Ser Ile Tyr Cys Met Pro Pro Arg Pro Cys Phe Lys Gly 790 795 Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp 810 Ala Leu Gly Ser Val Glu Ile Leu Leu Ser Arg His Cys Pro Ile Trp 825 Tyr Gly Tyr Asn Gly Arg Leu Lys Leu Leu Glu Arg Leu Ala Tyr Ile 840 Asn Thr Ile Val Tyr Pro Ile Thr Ser Ile Pro Leu Ile Ala Tyr Cys 855 860 Val Leu Pro Ala Ile Cys Leu Leu Thr Asn Lys Phe Ile Ile Pro Glu 870 875 Ile Ser Asn Tyr Ala Gly Met Phe Phe Ile Leu Leu Phe Ala Ser Ile 885 890 Phe Ala Thr Gly Ile Leu Glu Leu Arg Trp Ser Gly Val Gly Ile Glu 905 Asp Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Thr Ser Ala 920 His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala Gly Ile 940 Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu Asp Gly Asp 950 955

```
Phe Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser Leu Leu Ile Pro Pro
               965
                                  970
 Thr Thr Val Leu Val Ile Asn Leu Val Gly Met Val Ala Gly Ile Ser
                      985
            980
 Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys
                       1000
                                  1005
 Leu Phe Phe Ser Ile Trp Val Ile Leu His Leu Tyr Pro Phe Leu Lys
                             1020
                      1015
 Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp
 1025 1030
                           1035
 Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys Ile Asp
              1045
                      1050
 Pro Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala Leu Gly Gln Cys Gly
           1060 1065
 Val Asn Cys
       1075
      <210> 35
      <211> 25
      <212> DNA
      <213> Zea mays
      <400> 35
atggcggcca acaaggggat ggtgg
25
      <210> 36
      <211> 25
      <212> DNA
      <213> Zea mays
      <400> 36
tragragitg argreatatt greec
25
      <210> 37
      <211> 3969
      <212> DNA
      <213> Zea mays
      <220>
      <221> CDS
      <222> (144)...(3399)
      <400> 37
cttetecete gteggtgegg egtggegegg eteggegtte ggtgagaaac cacteggggg
atgaggatet getgetagag tgagaggage taeggteagt atectetgee ttegteggeg
                                                                120
geggaagteg aggggaggaa geg atg gag geg age gee ggg etg gtg gee gge
                                                                173
                       Met Glu Ala Ser Ala Gly Leu Val Ala Gly
tee cae aac ege aac gag ete gte gte ate ege ege gae gge gat eee
                                                                221
Ser His Asn Arg Asn Glu Leu Val Val Ile Arg Arg Asp Gly Asp Pro
                                  20
ggg ccg aag ccg ccg cgg gag cag aac ggg cag gtg tgc cag att tgc
                                                                269
Gly Pro Lys Pro Pro Arg Glu Gln Asn Gly Gln Val Cys Gln Ile Cys
            30
                              35
```

ggc	gac Asp	gac Asp 45	val	ggc . Gly	ctt Lei	geo Ala	ccc Pro	Gly	ggg Gly	gac Asp	e ccc	tto Phe 55	Va]	g gcg L Ala	tgc Cys	:	317
aac Asn	gag Glu 60	Cys	gcc Ala	tto Phe	e Pro	gto Val 65	Суз	cgg Arg	gac Asp	tgc Cya	tac Tyr 70	Glu	tac Tyr	gag Glu	cgc Arg	;	365
cgg Arg 75	Glu	ggc	acg Thr	cag Gln	Asn 80	. Cys	Pro	cag Gln	tgc Cys	aag Lys 85	Thr	cga Arg	tac Tyr	aag Lys	cgc Arg 90	4	113
ctc Leu	aag Lys	Gly	tgc Cys	caa Gln 95	Arg	gtg Val	acc Thr	ggt Gly	gac Asp 100	Glu	gag Glu	gag Glu	gac Asp	ggc Gly 105	gtc Val	4	161
gat Asp	gac Asp	ctg Leu	gac Asp 110	Asn	gag Glu	ttc Phe	aac Asn	tgg Trp 115	Asp	ggc	cat His	gac Asp	tcg Ser 120	Gln	tct Ser	5	509
gtg Val	gcc Ala	gag Glu 125	tcc Ser	atg Met	ctc Leu	tac Tyr	ggc Gly 130	cac His	atg Met	agc Ser	tac Tyr	ggc Gly 135	cgt Arg	gga Gly	ggt Gly	5	57
gac Asp	cct Pro 140	aat Asn	Gly	gcg Ala	cca Pro	caa Gln 145	gct Ala	ttc Phe	cag Gln	ctc Leu	aac Asn 150	ccc Pro	aat Asn	gtt Val	cca Pro	6	05
ctc Leu 155	ctc Leu	acc Thr	aac Asn	gly aaa	caa Gln 160	atg Met	gtg Val	gat Asp	gac Asp	atc Ile 165	cca Pro	ccg Pro	gag Glu	cag Gln	cac His 170	6	53
gcg Ala	ctg Leu	gtg Val	cct Pro	tct Ser 175	ttc Phe	atg Met	ggt Gly	ggt Gly	999 Gly 180	gga Gly	aag Lys	agg Arg	ata Ile	cat His 185	ccc Pro	7	01
ctt Leu	cct Pro	tat Tyr	gcg Ala 190	gat Asp	ccc Pro	agc Ser	tta Leu	cct Pro 195	gtg Val	caa Gln	ccc Pro	agg Arg	tct Ser 200	atg Met	gac Asp	7-	49
												gtt Val 215				7:	97
gaa Glu	cgg Arg 220	atg Met	gag Glu	aat Asn	tgg Trp	aag Lys 225	cag Gln	aga Arg	caa Gln	gag Glu	agg Arg 230	atg Met	cac His	cag Gln	acg Thr	84	45
999 Gly 235																89	93
atg Met			Ala													94	11
agc	cag	att	aat	cca	tat	agg	atg	att	atc	att	att	cgg	ctt	gtg	gtt	98	39

PCT/US99/18760

- 73 -

								- '								
Ser	Gln	Ile	Asn 270		Tyr	Arg	Met	Ile 275	Ile	Ile	Ile	Arg	Leu 280	Val	Val	
		ttc Phe 285														1037
		ttg Leu														1085
tct Ser 315	tgg Trp	att Ile	ctt Leu	gat Asp	caa Gln 320	ttc Phe	cca Pro	aag Lys	tgg Trp	ttc Phe 325	cct Pro	att Ile	gag Glu	aga Arg	gag Glu 330	1133
		cta Leu														1181
		ctt Leu														1229
		cct Pro 365								_					_	1277
		tat Tyr														1325
		atg Met														1373
		tgg Trp										_				1421
		tgg Trp				_	_		_		_		_	_		1469
-	-	aac Asn 445						_	_	-	_	_				1517
		aag Lys														1565
	_	gaa Glu				_		_								1613
	_	cgt Arg					_									1661

				gac Asp 510													1709
		_		aaa Lys	_							_		_		_	1757
				ttg Leu													1805
]				ttg Leu									_	_	_		1853
	_	-		atg Met	-		-	_	-					_	_	_	1901
				cag Gln 590				-					_	_		_	1949
	-		-	aac Asn			_	_			_			_			1997
	_	_		att Ile	-					-				-	_		2045
7	_		-	gca Ala					_	_		Lys		_	_		2093
				act Thr	_		_			_		-		_	_		2141
				aat Asn 670													2189
				tta Leu													2237
				ggt Gly													2285
I				att Ile													2333
t	ct	tct	gtt	ttt	gtt	aca	tcc	aca	ctt	ctc	gag	aat	ggt	gga	acc	ttg	2381

- 75 -

									,,							
Ser	Ser	· Val	. Phe	Val 735		Ser	Thr	Leu	Leu 740		Asn	Gly	Gly	745	Leu	
aag Lys	agt Ser	gca Ala	agt Ser 750	Pro	gct Ala	tct Ser	ctt Leu	ttg Leu 755	Lys	gaa Glu	gct Ala	ata Ile	cat His 760	Val	att Ile	2429
agt Ser	tgt Cys	ggt Gly 765	Tyr	gaa Glu	gac Asp	aag Lys	aca Thr 770	Asp	tgg Trp	gga Gly	aaa Lys	gag Glu 775	Ile	ggg	tgg Trp	2477
atc Ile	tat Tyr 780	Gly	tca Ser	gtt Val	aca Thr	gaa Glu 785	gat Asp	att Ile	cta Leu	act Thr	ggt Gly 790	Phe	aag Lys	atg Met	cat His	2525
tgt Cys 795	cat His	ggt Gly	tgg Trp	cgg Arg	tca Ser 800	att Ile	tac Tyr	tgc Cys	ata Ile	cct Pro 805	aaa Lys	cgg Arg	gtt Val	gca Ala	ttc Phe 810	2573
aaa Lys	ggt	tct Ser	gca Ala	cct Pro 815	ctg Leu	aat Asn	ctt Leu	tca Ser	gat Asp 820	cgt Arg	ctt Leu	cac His	cag Gln	gtg Val 825	ctt Leu	2621
cgg Arg	tgg Trp	gct Ala	ctt Leu 830	GJ A GG A	tct Ser	att Ile	gag Glu	atc Ile 835	ttc Phe	ttc Phe	agc Ser	aat Asn	cat His 840	tgc Cys	cct Pro	2669
			ggg Gly													2717
			tcc Ser													2765
tac Tyr 875	tgt Cys	aca Thr	ttg Leu	cct Pro	gcc Ala 880	atc Ile	tgt Cys	tta Leu	ttg Leu	aca Thr 885	gjà aaa	aaa Lys	ttt Phe	atc Ile	act Thr 890	2813
cca Pro	gag Glu	ctg Leu	aat Asn	aat Asn 895	gtt Val	gcc Ala	agc Ser	ctg Leu	tgg Trp 900	ttc Phe	atg Met	tca Ser	ctt Leu	ttt Phe 905	atc Ile	2861
tgc Cys	att Ile	ttt Phe	gct Ala 910	acg Thr	agc Ser	atc Ile	cta Leu	gaa Glu 915	atg Met	aga Arg	tgg Trp	agt Ser	ggt Gly 920	gtt Val	gga Gly	2909
			tgg Trp													2957
			ctc Leu													3005
ggt Gly 955	gtt Val	gat Asp	aca Thr	agc Ser	ttc Phe 960	acc Thr	gtg Val	aca Thr	tca Ser	aag Lys 965	ggt Gly	gga Gly	gat Asp	gat Asp	gag Glu 970	3053

gag ttc tca gag cta tat aca ttc aaa tgg act acc tta ttg ata cct Glu Phe Ser Glu Leu Tyr Thr Phe Lys Trp Thr Thr Leu Leu Ile Pro 975 980 985	3101
cct acc acc ttg ctt cta ttg aac ttc att ggt gtg gtc gct ggc gtt Pro Thr Thr Leu Leu Leu Leu Asn Phe Ile Gly Val Val Ala Gly Val 990 995 1000	3149
tca aat gcg atc aat aac gga tat gag tca tgg ggc ccc ctc ttt ggg Ser Asn Ala Ile Asn Asn Gly Tyr Glu Ser Trp Gly Pro Leu Phe Gly 1005 1010 1015	3197
aag cta ttc ttt gca ttt tgg gtg att gtc cat ctt tat ccc ttt ctc Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu 1020 1025 1030	3245
aaa ggt ttg gtt gga agg caa aac agg aca cca acg att gtc atc gtc Lys Gly Leu Val Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val 1035 1040 1045 1050	3293
tgg tcc att ctg ctg gct tca atc ttc tcg ctc ctt tgg gtt cgg att Trp Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Arg Ile 1055 1060 1065	3341
gat cct ttc ctt gcg aag gat gat ggt ccg ctt ctt gag gag tgt ggt Asp Pro Phe Leu Ala Lys Asp Asp Gly Pro Leu Leu Glu Glu Cys Gly 1070 1075 1080	3389
ttg gat tgc a actaggatgt cagtgcatca gctcccccaa tctgcatatg Leu Asp Cys 1085	3439
cttgaagtat attttctggt gtttgtcccc atattcagtg tctgtagata agagacatga aatgtcccaa gtttcttttg atccatggtg aacctactta atatctgaga gatatactgg gggaaaatgg aggctgcggc aatccttgtg cagttgggcc gtggaataca gcatatgcaa gtgtttgatt gtgcagcatt ctttattact tggtcgcaat atagatgggc tgagccgaac agcaaggtat tttgattctg cactgctccc gtgtacaaac ttggttctca ataaggcagg caggaatgca tctgccagtg gaacagagca acctgcacat tatttatgta tgcctgttca ttggagggct tgttcattac atgttcgtct atactagaaa aaacagaata ttagcattaa tctatagtta attaaagtat gtaaatgcgc ctgttttttg ttgtgtactg taatcatctg agttggtttt gtgaaaaaaa aaaaaaaaaa	3499 3559 3619 3679 3739 3799 3859 3919 3969
<210> 38 <211> 1086 <212> PRT <213> Zea mays	
<pre><400> 38 Met Glu Ala Ser Ala Gly Leu Val Ala Gly Ser His Asn Arg Asn Glu 1</pre>	
20 25 30 Glu Gln Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Asp Val Gly Leu	
35 40 45 Ala Pro Gly Gly Asp Pro Phe Val Ala Cys Asn Glu Cys Ala Phe Pro 50 55 60	
Val Cys Arg Asp Cys Tyr Glu Tyr Glu Arg Arg Glu Gly Thr Gln Asn	

PCT/US99/18760

- 77 -

```
70
                                       75
Cys Pro Gln Cys Lys Thr Arg Tyr Lys Arg Leu Lys Gly Cys Gln Arg
                                   90
Val Thr Gly Asp Glu Glu Glu Asp Gly Val Asp Asp Leu Asp Asn Glu
                              105
Phe Asn Trp Asp Gly His Asp Ser Gln Ser Val Ala Glu Ser Met Leu
                           120
Tyr Gly His Met Ser Tyr Gly Arg Gly Gly Asp Pro Asn Gly Ala Pro
                       135
                                           140
Gln Ala Phe Gln Leu Asn Pro Asn Val Pro Leu Leu Thr Asn Gly Gln
                   150
                                       155
Met Val Asp Asp Ile Pro Pro Glu Gln His Ala Leu Val Pro Ser Phe
                                   170
Met Gly Gly Gly Lys Arg Ile His Pro Leu Pro Tyr Ala Asp Pro
                              185
Ser Leu Pro Val Gln Pro Arg Ser Met Asp Pro Ser Lys Asp Leu Ala
                           200
                                               205
Ala Tyr Gly Tyr Gly Ser Val Ala Trp Lys Glu Arg Met Glu Asn Trp
                       215
                                          220
Lys Gln Arg Gln Glu Arg Met His Gln Thr Gly Asn Asp Gly Gly Gly
                   230
                                      235
Asp Asp Gly Asp Asp Ala Asp Leu Pro Leu Met Asp Glu Ala Arg Gln
                                   250
               245
Gln Leu Ser Arg Lys Ile Pro Leu Pro Ser Ser Gln Ile Asn Pro Tyr
                               265
Arg Met Ile Ile Ile Arg Leu Val Val Leu Gly Phe Phe Phe His
       275
                           280
Tyr Arg Val Met His Pro Val Asn Asp Ala Phe Ala Leu Trp Leu Ile
                       295
                                           300
Ser Val Ile Cys Glu Ile Trp Phe Ala Met Ser Trp Ile Leu Asp Gln
                   310
                                       315
Phe Pro Lys Trp Phe Pro Ile Glu Arg Glu Thr Tyr Leu Asp Arg Leu
               325
                                   330
Ser Leu Arg Phe Asp Lys Glu Gly Gln Pro Ser Gln Leu Ala Pro Ile
                               345
Asp Phe Phe Val Ser Thr Val Asp Pro Leu Lys Glu Pro Pro Leu Val
                           360
Thr Thr Asn Thr Val Leu Ser Ile Leu Ser Val Asp Tyr Pro Val Asp
                       375
                                           380
Lys Val Ser Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr Phe
                   390
                                       395
Glu Ala Leu Ser Glu Thr Ser Glu Phe Ala Lys Lys Trp Val Pro Phe
               405
                                   410
Cys Lys Arg Tyr Asn Ile Glu Pro Arg Ala Pro Glu Trp Tyr Phe Gln
                               425
Gln Lys Ile Asp Tyr Leu Lys Asp Lys Val Ala Ala Asn Phe Val Arg
                           440
Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile
                       455
Asn Ala Leu Val Ala Lys Ala Gln Lys Val Pro Glu Glu Gly Trp Thr
                                       475
Met Gln Asp Gly Thr Pro Trp Pro Gly Asn Asn Val Arg Asp His Pro
               485
                                   490
Gly Met Ile Gln Val Phe Leu Gly Gln Ser Gly Gly Leu Asp Cys Glu
                               505
Gly Asn Glu Leu Pro Arg Leu Val Tyr Val Ser Arg Glu Lys Arg Pro
                           520
Gly Tyr Asn His His Lys Lys Ala Gly Ala Met Asn Ala Leu Val Arg
```

535 Val Ser Ala Val Leu Thr Asn Ala Pro Tyr Leu Leu Asn Leu Asp Cys 550 Asp His Tyr Ile Asn Asn Ser Lys Ala Ile Lys Glu Ala Met Cys Phe 570 Met Met Asp Pro Leu Leu Gly Lys Lys Val Cys Tyr Val Gln Phe Pro 585 Gln Arg Phe Asp Gly Ile Asp Arg His Asp Arg Tyr Ala Asn Arg Asn 600 Val Val Phe Phe Asp Ile Asn Met Lys Gly Leu Asp Gly Ile Gln Gly 615 Pro Ile Tyr Val Gly Thr Gly Cys Val Phe Arg Arg Gln Ala Leu Tyr 635 Gly Tyr Asp Ala Pro Lys Thr Lys Lys Pro Pro Ser Arg Thr Cys Asn 645 650 Cys Trp Pro Lys Trp Cys Phe Cys Cys Cys Phe Gly Asn Arg Lys 665 Gin Lys Lys Thr Thr Lys Pro Lys Thr Glu Lys Lys Lys Leu Leu Phe 680 Phe Lys Lys Glu Glu Asn Gln Ser Pro Ala Tyr Ala Leu Gly Glu Ile 695 700 Asp Glu Ala Ala Pro Gly Ala Glu Asn Glu Lys Ala Gly Ile Val Asn 710 Gln Gln Lys Leu Glu Lys Lys Phe Gly Gln Ser Ser Val Phe Val Thr 730 Ser Thr Leu Leu Glu Asn Gly Gly Thr Leu Lys Ser Ala Ser Pro Ala 745 Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp 760 Lys Thr Asp Trp Gly Lys Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr 775 780 Glu Asp Ile Leu Thr Gly Phe Lys Met His Cys His Gly Trp Arg Ser 790 795 Ile Tyr Cys Ile Pro Lys Arg Val Ala Phe Lys Gly Ser Ala Pro Leu 805 810 Asn Leu Ser Asp Arg Leu His Gln Val Leu Arg Trp Ala Leu Gly Ser 825 Ile Glu Ile Phe Phe Ser Asn His Cys Pro Leu Trp Tyr Gly Tyr Gly 840 845 Gly Gly Leu Lys Phe Leu Glu Arg Phe Ser Tyr Ile Asn Ser Ile Val 855 860 Tyr Pro Trp Thr Ser Ile Pro Leu Leu Ala Tyr Cys Thr Leu Pro Ala 870 875 Ile Cys Leu Leu Thr Gly Lys Phe Ile Thr Pro Glu Leu Asn Asn Val 885 890 Ala Ser Leu Trp Phe Met Ser Leu Phe Ile Cys Ile Phe Ala Thr Ser 905 Ile Leu Glu Met Arg Trp Ser Gly Val Gly Ile Asp Asp Trp Trp Arg 920 Asn Glu Gln Phe Trp Val Ile Gly Gly Val Ser Ser His Leu Phe Ala 935 940 Val Phe Gln Gly Leu Leu Lys Val Ile Ala Gly Val Asp Thr Ser Phe 950 955 Thr Val Thr Ser Lys Gly Gly Asp Glu Glu Phe Ser Glu Leu Tyr 970 Thr Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Leu Leu Leu 985 Leu Asn Phe Ile Gly Val Val Ala Gly Val Ser Asn Ala Ile Asn Asn

WO 00/09706 PCT/US99/18760

- 79 -

1000 1005 995 Gly Tyr Glu Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe 1015 1020 Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys Gly Leu Val Gly Arg 1030 1035 Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp Ser Ile Leu Leu Ala 1045 1050 Ser Ile Phe Ser Leu Leu Trp Val Arg Ile Asp Pro Phe Leu Ala Lys 1060 1065 Asp Asp Gly Pro Leu Leu Glu Glu Cys Gly Leu Asp Cys Asn 1075 1080 <210> 39 <211> 25 <212> DNA <213> Zea mays <400> 39 atggaggcga gcgccgggct ggtgg 25 <210> 40 <211> 25 <212> DNA <213> Zea mays <400> 40 ctagttgcaa tccaaaccac actcc 25 <210> 41 <211> 3725 <212> DNA <213> Zea mays <220> <221> CDS <222> (179)...(3398) <400> 41 gcagcagcag caccaccact gcgcggcatt gcagcgagca agcgggaggg atctggggca 60 120 tggtggcggt cgctgccgct gccgctcgga tctagagggc cgcacgggct gattgccctc cgccggcctc gtcggtgtcg gtggagtgtg aatcggtgtg tgtaggagga gcgcggag 178 226 atg gcg gcc aac aag ggg atg gtg gca ggc tct cac aac cgc aac gag Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu 274 ttc gtc atg atc cgc cac gac ggc gac gcg cct gtc ccg gct aag ccc Phe Val Met Ile Arg His Asp Gly Asp Ala Pro Val Pro Ala Lys Pro acg aag agt gcg aat ggg cag gtc tgc cag att tgt ggc gac act gtt 322 Thr Lys Ser Ala Asn Gly Gln Val Cys Gln Ile Cys Gly Asp Thr Val 370 gge gtt tea gee act ggt gat gte ttt gtt gee tge aat gag tgt gee Gly Val Ser Ala Thr Gly Asp Val Phe Val Ala Cys Asn Glu Cys Ala 60 55 50

WO 00/09706

tto Phe 65	Pro	gto Val	tgc Cys	e cgc Arg	Pro 70	Сув	tat Tyr	gag Glu	tac Tyr	gag Glu 75	Arg	aag Lys	gaa Glu	ggg Gly	aac Asn 80	418
caa Gln	tgc Cys	tgc Cys	cct Pro	cag Gln 85	Суз	aag Lys	act Thr	aga Arg	tac Tyr 90	Lys	aga Arg	cag Gln	aaa Lys	ggt Gly 95	agc Ser	466
cct Pro	cga Arg	gtt Val	cat His 100	Gly	gat Asp	gat Asp	gag Glu	gag Glu 105	gaa Glu	gat Asp	gtt Val	gat Asp	gac Asp 110	Leu	gac Asp	514
aat Asn	gaa Glu	ttc Phe 115	aac Asn	tat Tyr	aag Lys	caa Gln	ggc Gly 120	aat Asn	gjà aaa	aag Lys	ggc	cca Pro 125	gag Glu	tgg Trp	cag Gln	562
		Gly	gat Asp													610
cac His 145	cat His	cgg Arg	att Ile	cca Pro	cgc Arg 150	ctt Leu	aca Thr	agt Ser	gga Gly	caa Gln 155	cag Gln	ata Ile	tct Ser	gga Gly	gag Glu 160	658
			gca Ala													706.
			gat Asp 180													754
			ttg Leu													802
			agc Ser													850
act Thr 225	aat Asn	aaa Lys	tat Tyr	cca Pro	gag Glu 230	gct Ala	aga Arg	gga Gly	gac Asp	atg Met 235	gag Glu	Gly ggg	act Thr	ggc Gly	tca Ser 240	898
			gat Asp													946
-			cca Pro 260				Asn	_				Tyr			_	994
			cgt Arg			Ile	_	_						_		1042
agt	cat	cca	gtg	cgt	aat	gct	tat	gga	ttg	tgg	cta	gta	tct	gtt	atc	1090

- 81 -

Ser	His 290	Pro	Val	Arg	Asn	Ala 295	Tyr	Gly	Leu	Trp	Leu 300	Val	Ser	Val	Ile	
tgt Cys 305	gag Glu	gtc Val	tgg Trp	ttt Phe	gcc Ala 310	ttg Leu	tcc Ser	tgg Trp	ctt Leu	cta Leu 315	gat Asp	cag Gln	ttc Phe	cca Pro	aaa Lys 320	1138
tgg Trp	tat Tyr	cca Pro	atc Ile	aac Asn 325	cgt Arg	gag Glu	aca Thr	tat Tyr	ctc Leu 330	gac Asp	agg Arg	ctt Leu	gca Ala	ttg Leu 335	agg Arg	1186
tat Tyr	gat Asp	aga Arg	gag Glu 340	gga Gly	gag Glu	cca Pro	tca Ser	cag Gln 345	ctg Leu	gct Ala	ccc Pro	att Ile	gat Asp 350	gtc Val	ttt . Phe	1234
gtc Val	agt Ser	aca Thr 355	gtg Val	gat Asp	cca Pro	ttg Leu	aag Lys 360	gaa Glu	cct Pro	cca Pro	ctg Leu	atc Ile 365	aca Thr	gcc Ala	aac Asn	1282
act Thr	gtt Val 370	ttg Leu	tcc Ser	att Ile	ctt Leu	gct Ala 375	gtg Val	gat Asp	tac Tyr	cct Pro	gtt Val 380	gac Asp	aaa Lys	gtg Val	tca Ser	1330
tgc Cys 385	tat Tyr	gtt Val	tct Ser	gat Asp	gat Asp 390	ggc Gly	tca Ser	gct Ala	atg Met	ctg Leu 395	act Thr	ttt Phe	gag Glu	tct Ser	ctc Leu 400	1378
tct Ser	gaa Glu	act Thr	gcc Ala	gaa Glu 405	ttt Phe	gct Ala	aga Arg	aag Lys	tgg Trp 410	gtt Val	ccc Pro	ttt Phe	tgt Cys	aag Lys 415	aag Lys	1426
cac His	aat Asn	att Ile	gaa Glu 420	cca Pro	aga Arg	gct Ala	cca Pro	gaa Glu 425	ttt Phe	tac Tyr	ttt Phe	gct Ala	caa Gln 430	aaa Lys	ata Ile	1474
gat Asp	tac Tyr	ctg Leu 435	aag Lys	gac Asp	aaa Lys	att Ile	caa Gln 440	cct Pro	tca Ser	ttt Phe	gtt Val	aag Lys 445	gaa Glu	aga Arg	cga Arg	1522
gca Ala	atg Met 450	aag Lys	aga Arg	gag Glu	tat Tyr	gaa Glu 455	gaa Glu	ttc Phe	aaa Lys	ata Ile	aga Arg 460	atc Ile	aat Asn	gcc Ala	ctt Leu	1570
gtt Val 465	Ala	aaa Lys	gca Ala	cag Gln	aaa Lys 470	gtg Val	cct Pro	gaa Glu	gag Glu	ggg Gly 475	tgg Trp	acc Thr	atg Met	gct Ala	gat Asp 480	1618
gga Gly	act Thr	gct Ala	tgg Trp	cct Pro 485	Gly	aat Asn	aac Asn	cct Pro	agg Arg 490	gac Asp	cat His	cct Pro	ggc Gly	atg Met 495	att Ile	1666
cag Gln	gtg Val	ttc Phe	ttg Leu 500	gly aaa	cac His	agt Ser	ggt Gly	999 61y 505	Leu	gac Asp	act Thr	gat Asp	gga Gly 510	aat Asn	gaa Glu	1714
tta Leu	cca Pro	cgt Arg 515	Leu	gtc Val	tat Tyr	gtc Val	tct Ser 520	Arg	gaa Glu	aag Lys	aga Arg	cca Pro 525	Gly	ttt Phe	cag Gln	1762

- 82 -

cat His	cac His 530	Lys	aag Lys	gct Ala	ggt	gca Ala 535	Met	aat Asn	gca Ala	. ctg . Leu	att Ile 540	Arg	gta Val	tct Ser	gct Ala	1810
gtg Val 545	Leu	aca Thr	aat Asn	ggt Gly	gcc Ala 550	tat Tyr	ctt Leu	ctc Leu	aat Asn	gtg Val 555	Asp	tgt Cys	gac	cat His	tac Tyr 560	1858
ttc Phe	aat Asn	agc Ser	agc Ser	aaa Lys 565	Ala	ctt Leu	aga Arg	gaa Glu	gca Ala 570	atg Met	tgc Cys	ttc Phe	atg Met	atg Met 575	gat Asp	1906
cca Pro	gct Ala	cta Leu	gga Gly 580	agg Arg	aaa Lys	act Thr	tgt Cys	tat Tyr 585	gta Val	caa Gln	ttt Phe	cca Pro	caa Gln 590	aga Arg	ttt Phe	1954
			gac Asp													2002
ttt Phe	gat Asp 610	atc Ile	aac Asn	atg Met	aaa Lys	ggt Gly 615	cta Leu	gat Asp	ggc Gly	att Ile	cag Gln 620	ggt Gly	cca Pro	gtc Val	tat Tyr	2050
gtg Val 625	gga Gly	aca Thr	gga Gly	tgc Cys	tgt Cys 630	ttc Phe	aat Asn	agg Arg	cag Gln	gct Ala 635	ttg Leu	tat Tyr	gga Gly	tat Tyr	gat Asp 640	2098
cct Pro	gtt Val	ttg Leu	act Thr	gaa Glu 645	gct Ala	gat Asp	ctg Leu	gaa Glu	cct Pro 650	aac Asn	att Ile	gtt Val	gtt Val	aag Lys 655	agc Ser	2146
			aga Arg 660													2194
			atg Met													2242
gaa Glu	gac Asp 690	atc Ile	gag Glu	gag Glu	ggt Gly	att Ile 695	gaa Glu	ggt Gly	tat Tyr	gag Glu	gat Asp 700	gaa Glu	agg Arg	tca Ser	gtg Val	2290
			cag Gln													2338
			tcc Ser													2386
			tct Ser 740													2434
tac	gag	gac	aaa	act	gaa	tgg	gga	aaa	gag	att	ggc	tgg	atc	tat	ggt	2482

- 83 -

Tyr	Glu	755		Thr	Glu	Trp	Gly 760		Glu	lle	e Gly	765		туг	Gly	
tca Ser	gtt Val 770	Thr	gag Glu	gat Asp	att Ile	ctg Leu 775	Thr	Gly ggg	ttt Phe	aaa Lys	atg Met 780	His	gca Ala	aga Arg	ggc Gly	2530
tgg Trp 785	Gln	tca Ser	atc Ile	tac Tyr	tgc Cys 790	Met	cca Pro	cca Pro	cga Arg	Pro 795	Cys	ttc Phe	aag Lys	ggt	tct Ser 800	2578
gca Ala	cca Pro	atc Ile	aat Asn	ctt Leu 805	Ser	gat Asp	cgt Arg	ctt Leu	aat Asn 810	Gln	gtg Val	ctc Leu	cgt Arg	tgg Trp 815	gct Ala	2626
ctt Leu	Gly aaa	tca Ser	gtg Val 820	gaa Glu	att Ile	ctg Leu	ctt Leu	agc Ser 825	Arg	cat His	tgt Cys	cct Pro	ata Ile 830	Trp	tat Tyr	2674
ggc Gly	tac Tyr	aat Asn 835	GJ y 999	cga Arg	ttg Leu	aag Lys	ctt Leu 840	ttg Leu	gag Glu	agg Arg	ctg Leu	gct Ala 845	tac Tyr	att Ile	aac Asn	2722
acc Thr	att Ile 850	gtt Val	tat Tyr	cca Pro	atc Ile	aca Thr 855	tct Ser	gtt Val	ccg Pro	ctt Leu	atc Ile 860	gcc Ala	tat Tyr	tgt Cys	gtg Val	2770
ctt Leu 865	cct Pro	gct Ala	atc Ile	tgt Cys	ctt Leu 870	ctt Leu	acc Thr	aat Asn	aaa Lys	ttt Phe 875	atc Ile	att Ile	cct Pro	gag Glu	att Ile 880	2818
agt Ser	aat Asn	tat Tyr	gct Ala	gga Gly 885	atg Met	ttc Phe	ttc Phe	att Ile	ctt Leu 890	ctt Leu	ttt Phe	gcc Ala	tcc Ser	att Ile 895	ttc Phe	2866
gca Ala	act Thr	ggt Gly	ata Ile 900	ttg Leu	gag Glu	ctc Leu	aga Arg	tgg Trp 905	agt Ser	ggt Gly	gtt Val	Gly	att Ile 910	gaa Glu	gat Asp	2914
tgg Trp	tgg Trp	aga Arg 915	aat Asn	gag Glu	cag Gln	ttt Phe	tgg Trp 920	gtt Val	att Ile	ggt Gly	ggc	acc Thr 925	tct Ser	gcc Ala	cat His	2962
Leu						ggt Gly 935										3010
acc Thr 945																3058
gct Ala			Tyr													3106
act f		Leu														3154

gcc att aac agc ggc tac caa tcc tgg ggt ccg ctc ttt gga aag ctg Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys Leu 995 1000 1005	3202
ttc ttc tcg atc tgg gtg atc ctc cat ctc tac ccc ttc ctc aag ggt Phe Phe Ser Ile Trp Val Ile Leu His Leu Tyr Pro Phe Leu Lys Gly 1010 1015 1020	3250
ctc atg ggc agg cag aac cgc acg cca aca atc gtc atc gtt tgg tcc Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp Ser 1025 1030 1035 1040	3298
atc ctc ctt gcg tct atc ttc tcc ttg ctg tgg gtg aag atc gat cct Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys Ile Asp Pro 1045 1050 1055	3346
ttc atc tcc ccg aca cag aaa gct gcc gcc ttg ggg caa tgt ggt gtg Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala Leu Gly Gln Cys Gly Val 1060 1065 1070	3394
aac t getgateeag attgtgaete ttatetgaag aggeteagee aaagatetge Asn	3448
cccctcgtgt aaatacctga gggggctaga tgggaatttt ttgttgtaga tgaggatgga	3500
totgoatoca agitatgoot otgittatta gottottogg tgooggtgot gotgoagaca	3508 3568
atcatggage etttetacet tgettgtagt getggecage agegtaaatt gtgaattetg	3628
catttttta tacgtggtgt ttattgtttt agagtaaatt atcatttgtt tgaggtaact attcacacga actatatggc aatgctgtta tttaaaa	3688
asserting accuracy accepted tetadad	3725
<210> 42	
<211> 1074	
<211> 1074 <212> PRT	
<211> 1074	
<211> 1074 <212> PRT <213> Zea mays <400> 42	
<pre><211> 1074 <212> PRT <213> Zea mays <400> 42 Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu 1 5 10 15</pre>	
<pre> <211> 1074 <212> PRT <213> Zea mays <400> 42 Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu 1</pre>	
<pre> <211> 1074 <212> PRT <213> Zea mays <400> 42 Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu 1</pre>	
<pre></pre>	

					16					1	70					17	5
				19	U				18	35					19	l As	p Pro
		1	195	•				20	00					20	p Tr	р Lу	s Glu
	4.1						23	L5					220	Me	t Le		n Val
22	⊋					23	U				2	35					y Ser 240
					24	5				2.5	0					25	u Ser
				201	,				26	5					27	g Ild	⊇ Val
		4	15					28	0.					285			J Ile
	49	U					29	5					300				l Ile
30	•					310)				3.	15					Lys 320
					325)				33	0					225	Arg
				340					34	5					350	1	. Phe
		3:	55					360	0					365			Asn
	3/1	,					37.	5					380				Ser
202	,					390					39	95					Leu 400
					405					410)					415	Lys
				420					425	;					430		Ile
		43	55					Glr 440)					445			
	450						455					_	160				
400						470		. Pro			47	5					490
					485			Asn		490						495	
			:	500				Gly	505						510		
		51	5					Ser 520						525			
	530						535	Met				5	40				
545						550		Leu			555	5					560
					565			Arg		570						575	-
			5	80				Cys	585						590		
		595	5					Arg 600					6	505			
	610						615	Leu				6:	20				
Val	Gly	Thi	G	ly (Cys	Cys	Phe	Asn	Arg	Gln	Ala	L	eu 1	yr (Gly	Tyr	qeA

- 86 -

										63.5					
625	77_7	T	TT la sa	a 1	630	>		~ 3	D	635	- 1 -	17- 1	777	T	640
PIO	vaı	Leu	Thr	645	ALA	Asp	Leu	GIU	650	ASII	тте	var	vaı	655	ser
Cva	Cve	Glv	Arg		Lva	Δνα	Tare	λαπ		Ser	Tur	Mot) en		Gln
Cys	Cys	GLy	660	ar 9	пуз	Ary	шyэ	665	כעם	UCI	- 7 -	rice	670	Jei	GIII
Ser	Ara	Ile	Met	Lvs	Ara	Thr	Glu	-	Ser	Ala	Pro	Ile		Asn	Met
	5	675		-,-	5		680					685			
Glu	Asp		Glu	Glu	Glv	Ile		Glv	Tyr	Glu	qzA		Arq	Ser	Val
	690					695			•		700		_		
Leu	Met	Ser	Gln	Arg	Lys	Leu	Glu	Lys	Arg	Phe	Gly	Gln	Ser	Pro	Ile
705				-	710			-	_	715	_				720
Phe	Ile	Ala	Ser	Thr	Phe	Met	Thr	Gln	Gly	Gly	Ile	Pro	Pro	Ser	Thr
				725					730					735	
Asn	Pro	Ala	Ser	Leu	Leu	Lys	Glu	Ala	Ile	His	Val	Iľe	Ser	Cys	Gly
			740				•	745					750		_
Tyr	Glu	_	Lys	Thr	Glu	Trp	_	Lys	Glu	Ile	Gly	_	Ile	Tyr	Gly
_		755		_		_	760			_		765	_ •	_	
Ser		Thr	Glu	Asp	Ile		Thr	GIY	Phe	Lys		His	Ala	Arg	GIY
(T)	770	C	71 -	Ma ess	~	775	Desa	D	7 mm	Dwa	780	Dha	T	~1	Co
785	GIII	Ser	Ile	Tyr	790	Met	PIO	PIO	Arg	795	Cys	Pne	nys	GIA	800
	Dro	Tla	Asn	T.011	-	Agn	Ara	T.e.u	Agn		Va 1	T.011	Δνα	Trn	
ALG		***	- TWII	805	561	дор	AL 9	DCu	810		741		9	815	nau
Leu	Glv	Ser	Val		Ile	Leu	Leu	Ser		His	Cvs	Pro	Ile		Tvr
	2		820	-				825			- 3 -		830		
Gly	Tyr	Asn	Gly	Arg	Leu	Lys	Leu	Leu	Glu	Arg	Leu	Ala	Tyr	Ile	Asn
_	_	835	_	_		_	840					845			
Thr	Ile	Val	Tyr	Pro	Ile	Thr	Ser	Val	Pro	Leu	Ile	Ala	Tyr	Cys	Val
	850					855					860				
Leu	Pro	Ala	Ile	Cys		Leu	Thr	Asn	Lys		Ile	Ile	Pro	Glu	
865					870	_	_		_	875			_		880
Ser	Asn	Tyr	Ala	_	Met	Phe	Phe	Ile		Leu	Phe	Ala	Ser		Phe
22-	mh	~ 3	- 1 -	885	~1	T	3	M	890	~ 1	17-1	a1	T1.0	895	N com
Ala	Thr	GIY	Ile	Leu	GIU	Leu	arg	905	ser	GLA	vaı	GIY	910	GIU	Asp
m	T~~	71 ***	900 Asn	G1.,	Gla	Dho	Tres		Tla	Glv	Glv	Thr		בומ	Нie
пр	пр	915	Maii	GIU	GIII	FIIC	920	vai	116	GLY	GLY	925	361	ΛIα	1110
T.e.ii	Dhe		Val	Phe	Gln	Glv		Len	Lvs	Val	T.e11		Glv	Ile	Asp
200	930		***			935	200		_,_		940		,		
Thr		Phe	Thr	Val	Thr		Lys	Ala	Ser	Asp	Glu	Asp	Gly	Asp	Phe
945					950		_			955		-	_	_	960
Ala	Glu	Leu	Tyr	Val	Phe	Lys	Trp					Ile	Pro	Pro	Thr
				965					970					975	
Thr	Val	Leu	Val	Ile	Asn	Leu	Val	Gly	Met	Val	Ala	Gly		Ser	Tyr
			980					985	_			_	990		_
Ala	Ile		Ser	Gly	Tyr	Gln			Gly	Pro	Leu			Lys	Leu
		995		_			100		_	_	_	100		T	61
Phe			Ile	Trp	Val			His	Leu	Tyr			Leu	ьys	GIA
	101			~1	•	101		D	mh	T1 -	102		1701	т	602
		GIÀ	Arg	GIN			Thr	Pro	Thr			TTE	AHI	пр	_
102		T	7 T ~	0.00	103		00	T 011	T 011	103		Lave	TIA	Ye.	1040 Pro
TTE	ьeц	neu	Ala	ser		Fue	aer	neu	105		val	пÄg	776	105	
Dha	Tle	Ser	Pro			Lve	Δls	Δla			Glv	Gln	Cva		
FIIE	116	261	106		GIII	Lys	A. d	106		LCU	O± y		107		
Agn	Cys		200	-					-				•		
	-10														

- 87 -

<210> 43 <211> 25 <212> DNA <213> Zea mays <400> 43 atggcggcca acaaggggat ggtgg 25 <210> 44 <211> 25 <212> DNA <213> Zea mavs <400> 44 tcagcagttc acaccacatt gcccc <210> 45 <211> 3813 <212> DNA <213> Zea mays <220> <221> CDS <222> (215)...(3494) <400> 45 ccacagetea tataccaaga geeggageag ettagegeag eccagagegg egeegeeca 60 agcacaaccc ccaccegcca cagcegegtg egeatgtgag eggtegeege ggeegggaga 120 ccagaggagg ggaggactac gtgcatttcg ctgtgccgcc gccgcggggt tcgtgcgcga 180 gcgagatccg gcggggcggg gcggggggcc tgag atg gag gct agc gcg ggg ctg 235 Met Glu Ala Ser Ala Gly Leu gtg gcc ggc tcg cat aac cgg aac gag ctg gtg gtg atc cgc cgc gac 283 Val Ala Gly Ser His Asn Arg Asn Glu Leu Val Val Ile Arg Arg Asp 10 15 cgc gag tcg gga gcc gcg ggc ggc gcg gcg cgc cgg gcg gag gcg 331 Arg Glu Ser Gly Ala Ala Gly Gly Gly Ala Ala Arg Arg Ala Glu Ala 30 ccg tgc cag ata tgc ggc gac gag gtc ggg gtg ggc ttc gac ggg gag 379 Pro Cys Gln Ile Cys Gly Asp Glu Val Gly Val Gly Phe Asp Gly Glu ccc ttc gtg gcg .tgc aac gag tgc gcc ttc ccc gtc tgc cgc gcc tgc 427 Pro Phe Val Ala Cys Asn Glu Cys Ala Phe Pro Val Cys Arg Ala Cys 475 tac gag tac gag cgc cgc gag ggc tcg caa gcg tgc ccg cag tgc agg Tyr Glu Tyr Glu Arg Arg Glu Gly Ser Gln Ala Cys Pro Gln Cys Arg acc cgc tac aag cgc ctc aag ggc tgc ccg cgg gtg gcc ggc gac gag 523 Thr Arg Tyr Lys Arg Leu Lys Gly Cys Pro Arg Val Ala Gly Asp Glu 90 95

· WO 00/09706

											ttc Phe 115					571
											gcc Ala			_		619
											gcg Ala					667
											ggc Gly					715
											tcc Ser					763
Gly											cct Pro 195					811
											tcc Ser					859
											aga Arg					907
											agc Ser					955
						_	_	_			atg Met	_	_	_		1003
											agc Ser 275					1051
		-			_			_		_	ttg Leu					1099
											ttt Phe					1147
											tcc Ser					1195
cag	ttc	cca	aag	tgg	ctt	cca	atc	gag	aga	gag	act	tac	ctg	gac	cgt	1243

- 89 -

Gln	Phe	Pro 330	Lys	Trp	Leu	Pro	Ile 335	Glu	Arg	Glu	Thr	Tyr 340	Leu	Asp	Arg	
					_	_	-				tct Ser 355	_		-		1291
											aag Lys					1339
											gtg Val			_	_	1387
						_		-	-		gct Ala	_	-		_	1435
											aag Lys					1483
											cct Pro 435					1531
	-			_		_		_	_	_	gct Ala	_			_	1579
					_	_	_				gaa Glu		_	-		1627
		-	_	_	_		_		_	_	cct Pro		_			1675
											aac Asn					1723
		-		_	_					_	ggc Gly 515					1771
_			_	_		_	_	_		_	tcg Ser	-	_	_		1819
											atg Met					1867
											cta Leu					1915

tgt Cys	gat Asp	cac His 570	tac Tyr	atc Ile	aac Asn	aat Asn	agc Ser 575	aag Lys	gcc Ala	ata Ile	aaa Lys	gag Glu 580	gct Ala	atg Met	tgt Cys	1963
ttc Phe	atg Met 585	atg Met	gat Asp	cct Pro	ttg Leu	gtg Val 590	GJA aaa	aag Lys	aaa Lys	gtg Val	tgc Cys 595	tat Tyr	gta Val	cag Gln	ttc Phe	2011
cct Pro 600	cag Gln	agg Arg	ttt Phe	gat Asp	ggt Gly 605	att Ile	gac Asp	aaa Lys	aat Asn	gat Asp 610	cga Arg	tac Tyr	gct Ala	aac Asn	agg Arg 615	2059
aac Asn	gtt Val	gtc Val	ttt Phe	ttt Phe 620	gac Asp	atc Ile	aac Asn	atg Met	aaa Lys 625	ggt Gly	ttg Leu	gac Asp	ggt Gly	att Ile 630	caa Gln	2107
gga Gly	ccc Pro	att Ile	tat Tyr 635	gtg Val	ggt Gly	act Thr	gga Gly	tgt Cys 640	gtt Val	ttc Phe	aga Arg	cgg Arg	cag Gln 645	gca Ala	ctg Leu	2155
tat Tyr	ggt Gly	tat Tyr 650	gat Asp	gct Ala	cct Pro	aaa Lys	acg Thr 655	aag Lys	aag Lys	cca Pro	cca Pro	tca Ser 660	aga Arg	act Thr	tgc Cys	2203
aac Asn	tgc Cys 665	tgg Trp	ccc Pro	aag Lys	tgg Trp	tgc Cys 670	ctc Leu	tct Ser	tgc Cys	tgc Cys	tgc Cys 675	agc Ser	agg Arg	aac Asn	aag Lys	2251
aat Asn 680	aaa Lys	aag Lys	aag Lys	act Thr	aca Thr 685	aaa Lys	cca Pro	aag Lys	acg Thr	gag Glu 690	aag Lys	aag Lys	aaa Lys	aga Arg	tta Leu 695	2299
					gaa Glu											2347
att Ile	gat Asp	gaa Glu	ggt Gly 715	gct Ala	cca Pro	ggt Gly	gct Ala	gat Asp 720	atc Ile	gag Glu	aag Lys	gcc Ala	gga Gly 725	atc Ile	gta Val	2395
					gag Glu											2443
					gag Glu											2491
					gaa Glu 765											2539
gac Asp	aag Lys	acc Thr	gac Asp	tgg Trp 780	gga Gly	aaa Lys	gag Glu	att Ile	ggc Gly 785	tgg Trp	att Ile	tac Tyr	gga Gly	tcg Ser 790	atc Ile	2587
aca	gag	gat	atc	ttg	act	gga	ttt	aag	atg	cac	tgc	cat	ggc	tgg	cgg	2635

- 91 -

Thr	Glu	Asp	Ile 795		Thr	Gly	Phe	Lys 800	Met	His	Cys	His	Gly 805	Trp	Arg	-
tct Ser	att Ile	tac Tyr 810	Cys	atc	ccg Pro	aag Lys	cgg Arg 815	cct Pro	gca Ala	ttc Phe	aaa Lys	ggt Gly 820	tct Ser	gcg Ala	cct Pro	2683
ctg Leu	aac Asn 825	ctt Leu	tcc Ser	gac Asp	cgt Arg	ctt Leu 830	cac His	cag Gln	gtc Val	ctt Leu	cgc Arg 835	tgg Trp	gcc Ala	ctt Leu	gjå aaà	2731
tcc Ser 840	gtc Val	gaa Glu	att Ile	ttc Phe	ttc Phe 845	agc Ser	aag Lys	cac His	tgc Cys	cca Pro 850	ctt Leu	tgg Trp	tac Tyr	gga Gly	tac Tyr 855	2779
ggc Gly	ggc Gly	Gly 999	cta Leu	aaa Lys 860	ttc Phe	ctg Leu	gaa Glu	agg Arg	ttt Phe 865	tct Ser	tat Tyr	atc Ile	aac Asn	tcc Ser 870	atc Ile	2827
gtt Val	tat Tyr	ccc Pro	tgg Trp 875	acg Thr	tcc Ser	att Ile	cct Pro	ctc Leu 880	ctg Leu	gct Ala	tac Tyr	tgt Cys	acc Thr 885	ttg Ļeu	cct Pro	2875
gcc Ala	atc Ile	tgc Cys 890	ctg Leu	ctc Leu	acg Thr	ggg Gly	aag Lys 895	ttt Phe	atc Ile	aca Thr	cca Pro	gag Glu 900	ctt Leu	acc Thr	aat Asn	2923
gtc Val	gcc Ala 905	agt Ser	atc Ile	tgg Trp	ttc Phe	atg Met 910	gca Ala	ctt Leu	ttc Phe	atc Ile	tgc Cys 915	atc Ile	tcc Ser	gtg Val	acc Thr	2971
ggc Gly 920	atc Ile	ctg Leu	gaa Glu	atg Met	agg Arg 925	tgg Trp	agt Ser	ggc Gly	gtg Val	gcc Ala 930	atc Ile	gac Asp	gac Asp	tgg Trp	tgg Trp 935	3019
agg Arg	aac Asn	gag Glu	cag Gln	ttc Phe 940	tgg Trp	gtc Val	atc Ile	gga Gly	ggc Gly 945	gtt Val	tcg Ser	gcg Ala	cat His	ctg Leu 950	ttc Phe	3067
gcg Ala	gtg Val	ttc Phe	cag Gln 955	ggc Gly	ctg Leu	ctg Leu	aag Lys	gtg Val 960	ttc Phe	gcc Ala	ggc Gly	atc Ile	gac Asp 965	acg Thr	agc Ser	3115
ttc Phe	acc Thr	gtg Val 970	acg Thr	tcg Ser	aag Lys	gcc Ala	999 Gly 975	gac Asp	gac Asp	gag Glu	gag Glu	ttc Phe 980	tcg Ser	gag Glu	ctg Leu	3163
tac Tyr	acg Thr 985	Phe	aag Lys	tgg Trp	acc Thr	acc Thr 990	Leu	ctg Leu	ata Ile	ccc Pro	ccg Pro 995	acc Thr	acg Thr	ctc Leu	ctc Leu	3211
ctg Leu 1000	Leu	aac Asn	ttc Phe	atc Ile	999 Gly 1005	Val	gtg Val	gcc Ala	gly ggg	atc Ile 1010	Ser	aac Asn	gcg Ala	atc Ile	aac Asn 1015	3259
aac Asn					Trp					Gly					Ala	3307

ttc tgg gt Phe Trp Va	g atc gtc l Ile Val 1035	cac ctg His Leu	tac ccg Tyr Pro 104	Phe Leu	aag ggt Lys Gly	ctg gtg ggg Leu Val Gly 1045	3355
Arg Gln As	c agg acg n Arg Thr 150	ccg acg Pro Thr	atc gtc Ile Val 1055	atc gtc Ile Val	tgg tcc Trp Ser 106	atc ctg ctg Ile Leu Leu O	3403
gcc tcg at Ala Ser Il 1065	c ttc tcg e Phe Ser	ctc ctg Leu Leu 1070	Trp Val	cgc gtc Arg Val	gac ccg Asp Pro 1075	ttc ctc gcc Phe Leu Ala	3451
aag agc aa Lys Ser As 1080	c ggc ccg n Gly Pro	ctc ctg Leu Leu 1085	gag gag Glu Glu	tgt ggc Cys Gly 1090	Leu Asp	tgc a Cys	3494
ttgttgttgt ctgctgtgtc gttttaaagt	tgttgttg cattggag tatacagt gacaaagg	ga atteti ca ggagag ga tgeaca ac atattg	tget gt gaggt ge attee ag	agatagaa ctgctgct tgcccagt	accacate gtttgttg gtattcc	tac gcctgatttt gtc cacggcatct gag taaattaaaa ctt tttacagtct taa aaaaaaaaa	3554 3614 3674 3734 3794 3813
<211 <212 <213	> 46 > 1094 > PRT > Zea may	's					
	> 46 a Ser Ala	Gly Leu	Val Ala	Gly Ser	His Asn	Arg Asn Glu	
1 Leu Val Va	5 1 Ile Arg 20	Arg Asp	Arg Glu 25	10 Ser Gly	Ala Ala	15 Gly Gly Gly 30	
Ala Ala Ar 35	g Arg Ala	Glu Ala		Gln Ile	Cys Gly	Asp Glu Val	
Gly Val Gl 50	y Phe Asp	Gly Glu 55	Pro Phe	Val Ala	Cys Asn 60	Glu Cys Ala	
Phe Pro Va 65	l Cys Arg	Ala Cys 70	Tyr Glu	Tyr Glu 75	Arg Arg	Glu Gly Ser 80	
Gln Ala Cy	s Pro Gln 85		Thr Arg	Tyr Lys	Arg Leu	Lys Gly Cys	
Pro Arg Va	l Ala Gly 100	Asp Glu	Glu Glu 105	Asp Gly	Val Asp	Asp Leu Glu	
Gly Glu Ph 11		Gln Asp		Ala His	Glu Asp 125	Asp Pro Gln	
Tyr Val Al 130	a Glu Ser	Met Leu 135	Arg Ala	Gln Met	Ser Tyr 140	Gly Arg Gly	
Gly Asp Al 145	a His Pro	Gly Phe 150	Ser Pro	Val Pro 155	Asn Val	Pro Leu Leu 160	
		Val Asp	Asp Ile	Pro Pro	Glu Gln	His Ala Leu	
Val Pro Se	165 r Tyr Met 180		Gly Gly 185	170 Gly Gly	Gly Lys	175 Arg Ile His	
Pro Leu Pr 19	o Phe Ala	Asp Pro		Pro Val	Gln Pro	190 Arg Ser Met	
Asp Pro Se							

- 93 -

Lys Glu Arg Met Glu Gly Trp Lys Gln Lys Gln Glu Arg Leu Gln His 230 235 Val Arg Ser Glu Gly Gly Gly Asp Trp Asp Gly Asp Asp Ala Asp Leu 245 250 Pro Leu Met Asp Glu Ala Arg Gln Pro Leu Ser Arg Lys Val Pro Ile 265 Ser Ser Ser Arg Ile Asn Pro Tyr Arg Met Ile Ile Val Ile Arg Leu 280 Val Val Leu Gly Phe Phe Phe His Tyr Arg Val Met His Pro Ala Lys 295 300 Asp Ala Phe Ala Leu Trp Leu Ile Ser Val Ile Cys Glu Ile Trp Phe 310 315 Ala Met Ser Trp Ile Leu Asp Gln Phe Pro Lys Trp Leu Pro Ile Glu 325 330 Arg Glu Thr Tyr Leu Asp Arg Leu Ser Leu Arg Phe Asp Lys Glu Gly 340 345 Gln Pro Ser Gln Leu Ala Pro Ile Asp Phe Phe Val Ser Thr Val Asp 360 Pro Thr Lys Glu Pro Pro Leu Val Thr Ala Asn Thr Val Leu Ser Ile 375 Leu Ser Val Asp Tyr Pro Val Glu Lys Val Ser Cys Tyr Val Ser Asp Asp Gly Ala Ala Met Leu Thr Phe Glu Ala Leu Ser Glu Thr Ser Glu 405 410 Phe Ala Lys Lys Trp Val Pro Phe Ser Lys Lys Phe Asn Ile Glu Pro 420 425 Arg Ala Pro Glu Trp Tyr Phe Gln Gln Lys Ile Asp Tyr Leu Lys Asp 440 Lys Val Ala Ala Ser Phe Val Arg Glu Arg Arg Ala Met Lys Arg Glu Tyr Glu Glu Phe Lys Val Arg Ile Asn Ala Leu Val Ala Lys Ala Gln 470 475 Lys Val Pro Glu Glu Gly Trp Thr Met Gln Asp Gly Ser Pro Trp Pro 490 Gly Asn Asn Val Arg Asp His Pro Gly Met Ile Gln Val Phe Leu Gly 505 Gln Ser Gly Gly Arg Asp Val Glu Gly Asn Glu Leu Pro Arg Leu Val 520 Tyr Val Ser Arg Glu Lys Arg Pro Gly Tyr Asn His His Lys Lys Ala 535 Gly Ala Met Asn Ala Leu Val Arg Val Ser Ala Val Leu Ser Asn Ala Ala Tyr Leu Leu Asn Leu Asp Cys Asp His Tyr Ile Asn Asn Ser Lys 565 570 Ala Ile Lys Glu Ala Met Cys Phe Met Met Asp Pro Leu Val Gly Lys 580 585 Lys Val Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Lys 600 Asn Asp Arg Tyr Ala Asn Arg Asn Val Val Phe Phe Asp Ile Asn Met 615 620 Lys Gly Leu Asp Gly Ile Gln Gly Pro Ile Tyr Val Gly Thr Gly Cys 630 635 Val Phe Arg Arg Gln Ala Leu Tyr Gly Tyr Asp Ala Pro Lys Thr Lys 645 650 Lys Pro Pro Ser Arg Thr Cys Asn Cys Trp Pro Lys Trp Cys Leu Ser 665 Cys Cys Cys Ser Arg Asn Lys Asn Lys Lys Lys Thr Thr Lys Pro Lys

- 94 -

Thr Glu Lys Lys Lys Arg Leu Phe Phe Lys Lys Ala Glu Asn Pro Ser 695 Pro Ala Tyr Ala Leu Gly Glu Ile Asp Glu Gly Ala Pro Gly Ala Asp 715 Ile Glu Lys Ala Gly Ile Val Asn Gln Gln Lys Leu Glu Lys Lys Phe 725 730 Gly Gln Ser Ser Val Phe Val Ala Ser Thr Leu Leu Glu Asn Gly Gly 740 745 Thr Leu Lys Ser Ala Ser Pro Ala Ser Leu Leu Lys Glu Ala Ile His 755 760 Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Asp Trp Gly Lys Glu Ile 775 780 Gly Trp Ile Tyr Gly Ser Ile Thr Glu Asp Ile Leu Thr Gly Phe Lys 790 795 Met His Cys His Gly Trp Arg Ser Ile Tyr Cys Ile Pro Lys Arg Pro 810 815 805 Ala Phe Lys Gly Ser Ala Pro Leu Asn Leu Ser Asp Arg Leu His Gln 820 825 Val Leu Arg Trp Ala Leu Gly Ser Val Glu Ile Phe Phe Ser Lys His 840 Cys Pro Leu Trp Tyr Gly Tyr Gly Gly Gly Leu Lys Phe Leu Glu Arg 855 860 Phe Ser Tyr Ile Asn Ser Ile Val Tyr Pro Trp Thr Ser Ile Pro Leu 870 875 Leu Ala Tyr Cys Thr Leu Pro Ala Ile Cys Leu Leu Thr Gly Lys Phe 885 890 Ile Thr Pro Glu Leu Thr Asn Val Ala Ser Ile Trp Phe Met Ala Leu 905 Phe Ile Cys Ile Ser Val Thr Gly Ile Leu Glu Met Arg Trp Ser Gly 920 Val Ala Ile Asp Asp Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly 935 Gly Val Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val 950 955 Phe Ala Gly Ile Asp Thr Ser Phe Thr Val Thr Ser Lys Ala Gly Asp 965 970 Asp Glu Glu Phe Ser Glu Leu Tyr Thr Phe Lys Trp Thr Thr Leu Leu 980 985 Ile Pro Pro Thr Thr Leu Leu Leu Asn Phe Ile Gly Val Val Ala 1000 1005 Gly Ile Ser Asn Ala Ile Asn Asn Gly Tyr Glu Ser Trp Gly Pro Leu 1015 1020 Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro 1025 1030 1035 Phe Leu Lys Gly Leu Val Gly Arg Gln Asn Arg Thr Pro Thr Ile Val 1045 1050 1055 Ile Val Trp Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val 1060 1065 1070 Arg Val Asp Pro Phe Leu Ala Lys Ser Asn Gly Pro Leu Leu Glu Glu 1075 1080 Cys Gly Leu Asp Cys Asn 1090

<210> 47 <211> 25

<212> DNA

<213> Zea mays

WO 00/09706 PCT/US99/18760

- 95 **-**

```
<400> 47
atggaggeta gegegggget ggtgg
     <210> 48
      <211> 25
      <212> DNA
      <213> Zea mays
      <400> 48
teagttgeag tecaggeeac actee
      <210> 49
      <211> 3746
      <212> DNA
      <213> Zea mays
      <220>
      <221> CDS
      <222> (321) . . . (3449)
      <400> 49
ctaggatcaa aaccgtctcg ccgctgcaat aatcttttgt caattcttaa tccctcgcgt
                                                                       60
egacagegae ageggaacea acteaegttg eegeggette etecateggt geggtgeeet
                                                                      120
gteettttet etegteeete eteeceegt atagttaage eeegeeege taetaetaet
                                                                      180
actagcagca gcagcgctct cgcagcggga gatgcggtgt tgatccgtgc cccgctcgga
                                                                      240
totogggact ggtgccggct ctgcccaggc cccaggctcc aggccagetc cctcgacgtt
                                                                      300
teteggegag etegettgee atg gag gge gae geg gae gge gtg aag teg ggg
                                                                      353
                       Met Glu Gly Asp Ala Asp Gly Val Lys Ser Gly
agg cgc ggt ggc gga cag gtg tgc cag atc tgc ggc gac ggc gtg ggc
                                                                      401
Arg Arg Gly Gly Gln Val Cys Gln Ile Cys Gly Asp Gly Val Gly
ace acg gcg gag ggg gac gtc ttc gcc gcc tgc gac gtc tgc ggg ttt
                                                                      449
Thr Thr Ala Glu Gly Asp Val Phe Ala Ala Cys Asp Val Cys Gly Phe
                             35
ccg gtg tgc cgc ccc tgc tac gag tac gag cgc aag gac ggc acg cag
                                                                      497
Pro Val Cys Arg Pro Cys Tyr Glu Tyr Glu Arg Lys Asp Gly Thr Gln
                          50
     45
                                                                      545
gcg tgc ccc cag tgc aag acc aag tac aag cgc cac aag ggg agc ccg
Ala Cys Pro Gln Cys Lys Thr Lys Tyr Lys Arg His Lys Gly Ser Pro
                                          70
 60
                      65
                                                                      593
ged atc egt ggg gag gaa gga gac gac act gat gec gat agc gac tte
Ala Ile Arg Gly Glu Glu Gly Asp Asp Thr Asp Ala Asp Ser Asp Phe
                                                          90
                  80
                                                                      641
aat tac ctt gca tct ggc aat gag gac cag aag cag aag att gcc gac
Asn Tyr Leu Ala Ser Gly Asn Glu Asp Gln Lys Gln Lys Ile Ala Asp
             95
                                 100
```

aga atg cgc agc tgg cgc atg aac gtt ggg ggc agc ggg gat gtt ggt Arg Met Arg Ser Trp Arg Met Asn Val Gly Gly Ser Gly Asp Val Gly 689

- 96 **-**

		110					115					120				
_		_		-	-						acc Thr 135	_		_	_	737
											act Thr					785
		_				-			_		cat His	_	_			833
					_	_	_				tat Tyr				_	881
		_							_		Gly 999		_	-		929
									-	_	gac Asp 215	_		_		977
											gag Glu					1025
	_		_	_			-			-	gaa Glu	-	_		_	1073
	-	_		-	-						gtt Val					1121
			Asn				_	Val			ctg Leu	-	_		_	1169
	_			_			-				cct Pro 295		_		-	1217
							-		_		atc Ile			-		1265
_			_	•	_			_			cca Pro					1313
											cgg Arg					1361

WO 00/09706 PCT/US99/18760

- 97 -

		ttg Leu 350														1409
aag Lys	gag Glu 365	cct Pro	cct Pro	ctt Leu	gtc Val	act Thr 370	gcc Ala	aat Asn	acc Thr	gtg Val	cta Leu 375	tcc Ser	att Ile	ctt Leu	gct Ala	1457
		tac Tyr														1505
		atg Met														1553
		tgg Trp														1601
		tgg Trp 430														1649
		tca Ser														1697
		aaa Lys														1745
		gaa Glu														1793
		mgg Xaa														1841
		ctt Leu 510														1889
		gaa Glu														1937
		gct Ala														1985
		aat Asn										_	_	_		2033
	-	gct Ala	_	_			_	_						_	_	2081

- 98 -

								_	•							
			575					580					585			
													agg Arg		-	2129
-		-								-			ttg Leu	_		2177
	_						_		_				tgt Cys	-		2225
													cag Gln			2273
							_						gca Ala 665			2321
		_		_	_	_	_	_	_	_	_		gtg Val	_	_	2369
													gtt Val			2417
													atg Met			2465
	_	-											act Thr			2513
							Ser		Thr				ctt Leu 745	Leu		2561
-	_			-		_	_				-	_	act Thr			2609
													gac Asp			2657
													tac Tyr			2705
													ctt Leu			2753

- 99 -

_	_		_			cgg Arg		-								2801
	-			-		ctg Leu									_	2849
	_		-			tac Tyr 850							_		_	2897
						tac Tyr										2945
		_				cca Pro			_			_	_			2993
						tcg Ser			_	_			_		-	3041
		_				atc Ile	-							_		3089
						tcc Ser 930										3137
						ggc										3185
						ggc										3233
						ccg Pro										3281
			Val			atc Ile		Tyr					Gly			3329
tcg Ser	tgg Trp 100	Gly	ccg Pro	ctc Leu	ttc Phe	ggc Gly 101	Lys	ctc Leu	ttc Phe	ttc Phe	gcc Ala 101	Phe	tgg Trp	gtc Val	atc Ile	3377
	His										Gly				cgc Arg 1035	3425
						gtc Val			atcc	tgc	tggc	gtcc	at c	ttct	ccttg	3479

- 100 -

ggca gcca cgta	atcaa atctg acaga	act o	gctag gtctg agtgg	gggaa gttaa gatat	ag to ag ti it gi	ggaag tatat ttac	gttt catat ccaca	gta ata	acttt agca	gta gca	gaaa agto	acgga ggcgt	agg a	aatad ttad	acgtgt cacgt cagcta ctcttt
	<2	210>	50												
		211>		3											
		212>		•											
	<2	213>	Zea	mays	3										
		100>	EΛ												
Met				Ala	Asp	ตา v	Val:	LVS	Ser	Glv	Ara	Δτα	Gl v	Gly	Glv
1		0-7		5		41		-,0	10	01,	9	9	O _T	15	U1,
	Val	Сув	Gln 20	Ile	Cys	Gly	Asp	Gly 25	Val	Gly	Thr	Thr	Ala 30	Glu	Gly
Asp	Val	Phe		Ala	Cys	Asp	Val	Cys	Gly	Phe	Pro	Val		Arg	Pro
_		35			-	_	40	-	_			45	•	•	
Cys	Tyr 50	Glu	Tyr	Glu	Arg	Lys 55	Asp	Gly	Thr	Gln	Ala 60	Cys	Pro	Gln	Сув
Lys	Thr	Lys	Tyr	Lys	Arg	His	Lys	Gly	Ser	Pro	Ala	Ile	Arg	Gly	Glu
65	_				70	_				75					80
	_	_	_	85	_		_		90			•		Ala 95	
Gly	Asn	Glu	Asp 100	Gln	Lys	Gln	Lys	Ile 105	Ala	Asp	Arg	Met	Arg 110	Ser	Trp
Arg	Met	Asn 115	Val	Gly	Gly	Ser	Gly 120	Asp	Val	Gly	Arg	Pro 125	Lys	Tyr	Asp
Ser	Gly 130	Glu	Ile	Gly	Leu	Thr 135	Lys	Tyr	Asp	Ser	Gly 140	Glu	Ile	Pro	Arg
Gly	Tyr	Ile	Pro	Ser	Val		Asn	Ser	Gln	Ile		Gly	Glu	Ile	Pro
145					150					155					160
Gly	Ala	Ser	Pro	Asp 165	His	His	Met	Met	Ser 170	Pro	Thr	Gly	Asn	Ile 175	Gly
Lys	Arg	Ala	Pro 180	Phe	Pro	Tyr	Val	Asn 185	His	Ser	Pro	Asn	Pro 190	Ser	Arg
Glu	Phe	Ser 195	Gly	Ser	Ile	Gly	Asn 200	Val	Ala	Trp	Lys	Glu 205	Arg	Val	Asp
Gly	Trp 210	Lys	Met	Lys	Gln	Asp 215	Lys	Gly	Thr	Ile	Pro 220	Met	Thr	Asn	Gly
Thr	Ser	Ile	Ala	Pro	Ser	Glu	Gly	Arg	Gly	Val	Gly	Asp	Ile	Asp	Ala
225					230					235					240
Ser	Thr	Asp	Tyr		Met	Glu	Asp	Ala		Leu	Asn	Asp	Glu	Thr	Arg
	_	_	_	245	_		_	_	250		_	_		255	
			260	_	•			265				_	270	Asn	
Tyr	Arg	Met 275	Val	Ile	Val	Leu	Arg 280	Leu	Ile	Val	Leu	Ser 285	Ile	Phe	Leu
His	Tyr 290	Arg	Ile	Thr	Asn	Pro 295	Val	Arg	Asn	Ala	Tyr 300	Pro	Leu	Trp	Leu
Leu 305		Val	Ile	Суз	Glu 310	Ile	Trp	Phe	Ala	Leu 315	Ser	Trp	Ile	Leu	Asp 320
	Phe	Pro	Lys	Trp 325		Pro	Ile	Asn	Arg 330		Thr	Tyr	Leu	Asp 335	
Leu	Ala	Leu	Arg 340		Asp	Arg	Glu	Gly 345		Pro	Ser	Gln	Leu 350	Ala	Ala

- 101 -

Val	Asp	Ile 355	Phe	Val	Ser	Thr	Val 360	Asp	Pro	Met	Lys	Glu 365	Pro	Pro	Leu
Val	Thr 370	Ala	Asn	Thr	Val	Leu 375	Ser	Ile	Leu	Ala	Val 380	Asp	Tyr	Pro	Val
Asp	Lys	Val	Ser	Суs	Tyr	Val	Ser	Asp	Asp	Gly	Ala	Ala	Met	Leu	Thr
385	-			_	390					395					400
Phe	Δsn	Ala	Len	Ala	Glu	Thr	Ser	Glu	Phe	Ala	Ara	Lvs	Trp	Val	Pro
				405					410		3	-1-		415	
Dha	3703	T	T		7.00	710	C1.11	Dra		717	Dro	C1	т		Dho
Pue	vai	гуя	-	TAT	ASII	TIE	GIU		Arg	ALG	PIO	GIU	Trp	TAT	PILE
	_		420			_	_	425	_			_	430		
Ser	Gln	Lys	Ile	Asp	Tyr	Leu	-	Asp	Lys	Val	His	Pro	Ser	Phe	Val
		435					440					445			
Lys	Asp	Arg	Arg	Ala	Met	Lys	Arg	Glu	Tyr	Glu	Glu	Phe	Lys	Val	Arg
	450					455					460				
Val	Asn	Glv	Leu	Val	Ala	Lys	Ala	Gln	Lys	Val	Pro	Glu	Glu	Gly	Trp
465		•			470	•			-	475				•	480
	Met	Gln	Δan	Glv		Pro	Trn	Pro	Glv		Asn	Thr	Xaa	Asn	
116	Mec	GIII	rop	485	****		1-5		490		77.011	1111	2144	495	
D	a1	14	T1 -		17-7	The o	T	<u>ما</u>		C	~1	41	T		mh-
Pro	GTA	мес		GIII	var	hue	ьец		птэ	ser	GTÀ	GIY	Leu	Asp	IIIL
			500					505		_			510		
Glu	Gly	Asn	Glu	Leu	Pro	Arg	Leu	Val	Tyr	Val	Ser	Arg	Glu	Lys	Arg
		515					520					525			
Pro	Gly	Phe	Gln	His	His	Lys	Lys	Ala	Gly	Ala	Met	Asn	Ala	Leu	Val
	530					535	_		_		540				
Ara	-	Ser	Ala	Val	Leu	Thr	Asn	Glv	Gln	Tvr	Met	Leu	Asn	Leu	Asp
545					550			1	•	555					560
	7 cn	Ti c	The res	T10		7 cn	Sar	Tara	71 a) roi	Glu	Ala	Mot	-
Cys	ASP	urs	IÀT		ASII	ASII	261	тåъ		Беи	Arg	GIU	ALA		Cys
_			_	565	_	_		_	570		_	_		575	
Phe	Leu	Met	Asp	Pro	Asn	Leu	Gly		Ser	Val	Cys	Tyr	Val	Gln	Phe
			580					585					590		
Pro	Gln	Arg	Phe	Asp	Gly	Ile	qaA	Arg	Asn	Asp	Arg	Tyr	Ala	Asn	Arg
		595					600					605			
Asn	Thr	Val	Phe	Phe	Asp	Ile	Asn	Leu	Arg	Gly	Leu	Asp	Gly	Ile	Gln
	610				_	615					620				
Glv	Pro	Val	Tvr	Val	Glv	Thr	Glv	Cvs	Val	Phe	Asn	Ara	Thr	Ala	Leu
625			-1-		630		3	-4		635					640
	G1 v	Тч гэ	Gl.	Pro		Tla	Lve	Gla	Tare		Glv	G1 v	Phe	T.611	
TYL	GLY	TYL	GIU	645	110	116	בינם	9111	650	_,.	013	017		655	
	. .	-	~7		•	.	v	.1.		T	0	T	T		0
ser	Leu	Сув	_	GTA	AIG	гÀа	гÀЗ		ser	гåа	ser	цув	Lys	GIY	ser
			660					665		_	_	.	670		
Asp	Lys	Lys	Lys	Ser	Gln	Lys		Val	Asp	Ser	Ser		Pro	Val	Pne
		675					680					685			
Asn	Leu	Glu	Asp	Ile	Glu	Glu	Gly	Val	Glu	Gly	Ala	Gly	Phe	Asp	Asp
	690					695					700				
Glu	Lys	Ser	Leu	Leu	Met	Ser	Gln	Met	Ser	Leu	Glu	Lys	Arg	Phe	Gly
705	-				710					715		-			720
		Δla	Δla	Phe			Ser	Thr	Leu	Met	Glu	Tvr	Gly	Glv	Val
0111	DCI	7.1.4		725					730			-1-	,	735	
D	~ 3	0	31.			G1	C	T 011			C1 11	71-	Tla		17 a 3
Pro	GIN	ser			PIO	GIU	Ser			пуз	Gru	ALG	Ile	пто	VAI
	٠		740		_		_	745		_			750		
Ile	Ser	Cys	Gly	Tyr	Glu	Asp			Glu	Trp	GIA		Glu	lie	GTA
		755					760					765			
Trp	Ile	Tyr	Gly	Ser	Val	Thr	Glu	Asp	Ile	Leu	Thr	Gly	Phe	Lys	Met
-	770		_			775					780				
His			Glv	Trp	Ara	Ser	Ile	Tyr	Cys	Met	Pro	Lys	Arg	Pro	Ala
785			1		790			-	-	795		_	_		800
		Glv	Ser	Δla			Asn	Len	Ser			Leu	Asn	Gln	
5.116	- Lys	y	JUL	805					810		5			815	
				603					020						

- 102 -

```
Leu Arg Trp Ala Leu Gly Ser Val Glu Ile Leu Phe Ser Arg His Cys
                          825
Pro Leu Trp Tyr Gly Tyr Gly Gly Arg Leu Lys Phe Leu Glu Arg Phe
                      840
Ala Tyr Ile Asn Thr Thr Ile Tyr Pro Leu Thr Ser Ile Pro Leu Leu
                 855
                                  860
Ile Tyr Cys Ile Leu Pro Ala Ile Cys Leu Leu Thr Gly Lys Phe Ile
        870
                        875
Ile Pro Glu Ile Ser Asn Phe Ala Ser Ile Trp Phe Ile Ser Leu Phe
                     890
Ile Ser Ile Phe Ala Thr Gly Ile Leu Glu Met Arg Trp Ser Gly Val
         900 905
Gly Ile Asp Glu Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly
                      920
Ile Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu
                   935
Ala Gly Ile Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu
       950
                                 955
Asp Gly Asp Phe Ala Glu Leu Tyr Met Phe Lys Trp Thr Thr Leu Leu
                              970
            965
Ile Pro Pro Thr Thr Ile Leu Ile Ile Asn Leu Val Gly Val Val Ala
         980 985
Gly Ile Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu
                              1005
              1000
Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro
   1010 1015
Phe Leu Lys Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val
Val Val Trp
```

<210> 51

<211> 25

<212> DNA

<213> Zea mays

<400> 51

atggagggcg acgcggacgg cgtga 25

<210> 52

<211> 25

<212> DNA

<213> Zea mays

<400> 52

ctageagttg atgecaeacg tetgg

<210> 53

<211> 3753

<212> DNA

<213> Zea mays

<220>

<221> CDS

<222> (184) ... (3406)

- 103 -

<400> 53

	<4	00>	53													
_	_	_	_				-			_		-	_		ca tg gt	60
	_	_				_		_		-	_			_	gcctc	120
							-							-	gagcg	180
gag	atg															228
	Met	Ala	Ala	Asn	Lys	Gly	Met	Val	Ala	Gly	Ser	His	Asn	Arg	Asn	
	1				5					10					15	
gag	ttc	gtc	atg	atc	cgc	cac	gac	ggc	gat	gtg	ccg	ggc	tcg	gct	aag	276
Glu	Phe	Val	Met	Ile	Arg	His	Asp	Gly	Asp	Val	Pro	Gly	Ser	Ala	Lys	
				20					25					30		
CCC	aca	aag	agt	aca	aat	gga	cag	gtc	tgc	cag	att	tgc	ggt	gac	tct	324
Pro	Thr	Lys	Ser	Ala	Asn	Gly	Gln	Val	Cys	Gln	Ile	Суз	Gly	Asp	Ser	
			35					40					45			
	ggt															372
Val	Gly		Ser	Ala	Thr	Gly		Val	Phe	Val	Ala	_	Asn	Glu	Cys	
		50					55					60				
_	ttc		-	-	_		_					-	_			420
Ala	Phe	Pro	Val	Cys	Arg		Cys	Tyr	GLu	Tyr		Arg	Lys	Glu	Gly	
	65					70					75					
	caa	-	_		-	_	_		-		_	_	_			468
	Gln	Cys	Cys	Pro		Cys	Lys	Thr	Arg		Lys	Arg	GIn	Lys		
80					85					90					95	
•	cct	_	_			_		_		_	_	_	_	_		516
Ser	Pro	Arg	vaı		GIA	Asp	GIu	Asp		GIU	Asp	vaı	Asp		Leu	
				100					105					110		
																564
-	aat	-				_			_							564
Asp	Asn	GIU		ASI	Tyr	гÀв	GIN		ser	GIY	гàг	GIY		GIU	Пр	
			115					120					125			
	ctg		~~~	~a+	~a+	aat	~a+	a+a	+ ~+	tas	+ a+	~~+	~~~	cat	~ 2~	612
	Leu															012
GIII	TIER	130	GLY	wab	ASP	ALA	135	nea	per	Ser	Ser	140	Arg	mra	GIU	
		130					133					140				
002	cat	cat	caa	2++	cca	cac	cta	202	age	aat	caa	cad	ata	tet	aas	660
	His															
FIU	145	*****	~ y	110	110	150	пси	****	DCI	GLy	155	0111	++0	001	OL y	
	110		•													
aaa	att	cct	gat	act	tcc	cct	gac	cat	cat	tet	atc	cac	agt	cca	aca	708
	Ile															•
160					165			9		170		5			175	
tea	agc	tat	att	gat	cca	адс	atc	cca	att	cct	ata	ада	att	ata	gac	756
_	Ser		_	_		_	_		_						-	
502		- , -		180				•••	185			3		190	F	
ccc	tcg	aac	gac	tta	aat	tee	tat	aaa	att	aat	aσt	att	gac	taa	aaσ	804
	Ser															
		,	195				- z -	200					205		•	
								_ • •								
gaa	aga	att	σaσ	age	taa	agg	att	aaa	caq	gac	aaa	aat	atg	atg	caa	852
	Arg															
	3				- ~ =					- 1	4					

- 104 -

		210					215					220				
gtg Val	act Thr 225	aat Asn	aaa Lys	tat Tyr	cca Pro	gag Glu 230	gct Ala	aga Arg	gga Gly	gga Gly	gac Asp 235	atg Met	gag Glu	ggg Gly	act Thr	900
											gat Asp					948
											ctc Leu					996
											ttc Phe					1044
cgt Arg	gtc Val	agt Ser 290	cat His	cca Pro	gtg Val	cgt Arg	gat Asp 295	gct Ala	tat Tyr	gga Gly	tta Leu	tgg Trp 300	cta Leu	gta Val	tct Ser	1092
											ctt Leu 315					1140
											ctt Leu					1188
							_			-	ctg Leu	_			-	1236
											cct Pro		_			1284
					Ser		Leu	Ser			tac Tyr					1332
											atg Met 395					1380
											tgg Trp					1428
_	_				_		-	_		_	ttt Phe			_		1476
											tca Ser					1524

- 105 -

aga Arg	cgc Arg	gca Ala 450	Met	aag Lys	agg Arg	gag Glu	tat Tyr 455	Glu	gaa Glu	ttc Phe	aaa Lys	gta Val 460	aga Arg	atc	aat Asn	1572
gcc Ala	Leu 465	Val	gcc Ala	aaa Lys	gca Ala	cag Gln 470	aaa Lys	gtg Val	cct Pro	gaa Glu	gag Glu 475	GJ Y	tgg Trp	acc Thr	atg Met	1620
gct Ala 480	gat Asp	gga Gly	act Thr	gca Ala	tgg Trp 485	cct Pro	GJ y aga	aat Asn	aat Asn	cct Pro 490	agg Arg	gac Asp	cat His	cct Pro	ggc Gly 495	1668
atg Met	att Ile	cag Gln	gtt Val	ttc Phe 500	ttg Leu	Gly 999	cac	agt Ser	ggt Gly 505	Gly aaa	ctc Leu	gac Asp	act Thr	gat Asp 510	gga Gly	1716
Asn	gag Glu	Leu	Pro 5 1 5	Arg	Leu	Val	Tyr	Val 520	Ser	Arg	Glu	Lys	Arg 525	Pro	Gly	1764
ttt Phe	cag Gln	cat His 530	cac His	aag Lys	aag Lys	gct Ala	ggt Gly 535	gca Ala	atg Met	aat Asn	gcg Ala	ctg Leu 540	att Ile	cgt Arg	gta Val	1812
tct Ser	gct Ala 545	gtg Val	ctg Leu	aca Thr	aat Asn	ggt Gly 550	gcc Ala	tat Tyr	ctt Leu	ctc Leu	aat Asn 555	gtg Val	gat Asp	tgc Cys	gac Asp	1860
cat His 560	tac Tyr	ttc Phe	aat Asn	agc Ser	agc Ser 565	aaa Lys	gct Ala	ctt Leu	aga Arg	gaa Glu 570	gca Ala	atg Met	tgc Cys	ttc Phe	atg Met 575	1908
atg Met	gat Asp	ccg Pro	gct Ala	cta Leu 580	gga Gly	agg Arg	aaa Lys	act Thr	tgt Cys 585	tat Tyr	gta Val	caa Gln	ttt Phe	cca Pro 590	cag Gln	1956
, aga Arg	ttt Phe	gat Asp	ggc Gly 595	att Ile	gac Asp	ttg Leu	cac His	gat Asp 600	cga Arg	tat Tyr	gct Ala	aat Asn	cgg Arg 605	aac Asn	ata Ile	2004
Val	ttc Phe	Phe 610	Asp	Ile	Asn	Met	Lys 615	Gly	Leu	Asp	Gly	Ile 620	Gln	Gly	Pro	2052
gtt Val	tac Tyr 625	gtg Val	gga Gly	aca Thr	gga Gly	tgc Cys 630	tgt Cys	ttc Phe	aat Asn	aga Arg	cag Gln 635	gct Ala	ttg Leu	tat Tyr	gga Gly	2100
tac Tyr 640	gat Asp	cct Pro	gtt Val	ttg Leu	act Thr 645	gaa Glu	gct Ala	gat Asp	ctg Leu	gag Glu 650	cca Pro	aac Asn	att Ile	gtt Val	att Ile 655	2148
aag Lys	agc Ser	tgc Cys	tgt Cys	ggt Gly 660	aga Arg	agg Arg	aag Lys	aaa Lys	aag Lys 665	aac Asn	aag Lys	agt Ser	Tyr	atg Met 670	gat Asp	2196
agt Ser	caa Gln	agc Ser	cgt Arg	att Ile	atg Met	aag Lys	aga Arg	aca Thr	gaa Glu	tct Ser	tca Ser	gct Ala	ccc Pro	atc Ile	ttc Phe	2244

- 106 -

675	680		685
aat atg gaa gac atc Asn Met Glu Asp Ile 690			
tca gtg ctt atg tcc Ser Val Leu Met Ser 705			
cct att ttc att gca Pro Ile Phe Ile Ala 720			
tca aca aac cca gct Ser Thr Asn Pro Ala 740	Ser Leu Leu Lys		
tgt gga tat gag gad Cys Gly Tyr Glu Asp . 755		Gly Lys Glu Ile	
tat ggt tca gta acg Tyr Gly Ser Val Thr 770			
agg ggc tgg caa tca Arg Gly Trp Gln Ser 785			
ggt tot gca cca ato Gly Ser Ala Pro Ile 800			
tgg gct ctt ggg tca Trp Ala Leu Gly Ser 820	Val Glu Ile Leu		
tgg tat ggt tac aat Trp Tyr Gly Tyr Asr 835	n Gly Arg Leu Lys	g ctt ttg gag agg g Leu Leu Glu Arg	Leu Ala Tyr
atc aac act att gta Ile Asn Thr Ile Val 850	tat cca atc aca Tyr Pro Ile Thr 855	a tcc att ccg ctt c Ser Ile Pro Leu 860	att gcc tat 2772 Ile Ala Tyr
tgt gtg ctt ccc gct Cys Val Leu Pro Ala 865			
gag att agc aat tat Glu Ile Ser Asn Tyr 880	c gct ggg atg tto c Ala Gly Met Phe 885	e ttc att ctt ctt e Phe Ile Leu Leu 890	ttc gcc tcc 2868 Phe Ala Ser 895
att ttt gcc act gg Ile Phe Ala Thr Gl	y Ile Leu Glu Le	t aga tgg agt ggt u Arg Trp Ser Gly 905	gtt ggc att 2916 Val Gly Ile 910

- 107 -

gaa gat tgg tgg aga aat gag cag ttt tgg gtt att ggt ggc acc Glu Asp Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Thr 915 920 925	
gcc cat ctc ttc gca gtg ttc cag ggt ctg ctg aaa gtg ttg gct Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala 930 935 940	ggg 3012 Gly
att gat acc aac ttc aca gtt acc tca aag gca tct gat gag gat ille Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu Asp 945 950 955	Gly ggc 3060
gac ttt gct gag cta tat gtg ttc aag tgg acc agt ttg ctc att Asp Phe Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser Leu Leu Ile 960 965 970	
ccg acc act gtt ctt gtc att aac ctg gtc gga atg gtg gca gga Pro Thr Thr Val Leu Val Ile Asn Leu Val Gly Met Val Ala Gly 980 985 990	att 3156 Ile
Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe 995 1000 1005	
aag ctg ttc ttc tcg atc tgg gtg atc ctc cat ctc tac ccc ttc Lys Leu Phe Phe Ser Ile Trp Val Ile Leu His Leu Tyr Pro Phe : 1010 1015 1020	
aag ggt ctc atg gga agg cag aac cgc aca cca aca atc gtc att c Lys Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile 1025 1030 1035	
tgg tcc atc ctt ctt gca tct atc ttc tcc ttg ctg tgg gtg aag atc. Trp Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys 1040 1045 1050	
gat cet tte ate tee eeg aca cag aaa get get gee ttg ggg caa a Asp Pro Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala Leu Gly Gln 1060 1065 1070	Cys
ggc gtc aac t gctgatcgag acagtgactc ttatttgaag aggctcaatc Gly Val Asn	3446
aagatctgcc ccctcgtgta aatacctgag gaggctagat gggaattcct tttgtggtgaggatgg atttgcatct aagttatgcc tctgttcatt agcttcttcc gtgccgctgctgcggac taagaatcac ggagcctttc taccttccat gtagcgccag ccagcaagatgtgaa ttttgaagtt ttgttatgcg tgcagtttat tgttttagag taaatttttgtttgtg ggaactgttc acacgagctt ataatggcaa tgctgttatt taaaaaaaaaa	ggtgc 3566 agcgt 3626 tatca 3686

<210> 54

<211> 1075

<212> PRT

<213> Zea mays

<400> 54

Met Ala Ala Asn Lys Gly Met Val Ala Gly Ser His Asn Arg Asn Glu

- 108 -

1				5					10					15	
	Val	Met	Ile 20	_	His	Asp	Gly	Asp 25		Pro	Gly	Ser	Ala 30	Lys	Pro
Thr	Lys	Ser 35	Ala	Asn	Gly	Gln	Val 40	Cys	Gln	Ile	Cys	Gly 45	Asp	Ser	Val
	50					55					60			Cys	
65					70					75				Gly	80
	_			85					90					Gly 95	
			100					105					110	Leu	
		115		_	_		120					125		Trp	
	130					135					140			Glu	
145					150					155				Gly	160
				165					170					Thr 175	
			180					185					190	Asp	
	_	195					200					205		Lys	
	210					215					220			Gln	
225		_	_		230					235				Thr	240
				245					250					Pro 255	
			260					265					270	Arg	
		275					280					285		Tyr	
	290					295					300			Ser Phe	
305	Cys	GIU	vai	пр	310	MIG	пеп	361	ııp	315	Dea	wop		• •••	320
	Trp	Tyr	Pro	Ile 325		Arg	Glu	Thr	Tyr 330	Leu	Asp	Arg	Leu	Ala 335	Leu
			340					345					350		
		355					360					365		Thr	
	370					375					380			ŗÀa	
385					390					395				Glu	400
				405					410					Cys 415	
			420					425					430		
		435					440	1				445	i	Glu	
	450	1				455	5				460	1		Asn	
Leu	. Val	Ala	Lys	Ala	Gln	Lys	val	. Pro	Glu	Glu	Gly	Trp	Thr	Met	. Ala

465															
465					470					475					480
Asp	Gly	Thr	Ala	Trp 485	Pro	Gly	Asn	Asn	Pro 490	Arg	Asp	His	Pro	Gly 495	Met
Ile	Gln	Val	Phe 500	Leu	Gly	His	Ser	Gly 505	Gly	Leu	Asp	Thr	Asp 510	Gly	Asn
Glu	Leu	Pro 515	Arg	Leu	Val	Tyr	Val 520	Ser	Arg	Glu	Lys	Arg 525	Pro	Gly	Phe
Gln	His 530	His	Lys	Lys	Ala	Gly 535	Ala	Met	Asn	Ala	Leu 540	Ile	Arg	Val	Ser
Ala 545	Val	Leu	Thr	Asn	Gly 550	Ala	Tyr	Leu	Leu	Asn 555	Val	Asp	Cys	Asp	His 560
Tyr	Phe	Asn	Ser	Ser 565	Lys	Ala	Leu	Arg	Glu 570	Ala	Met	Cys	Phe	Met 575	Met
_			Leu 580	_	_	_		585					590		-
	_	595	Ile	_			600		_			605			
	610	_	Ile			615	_		_	•	620		-		
625		_	Thr		630	_				635				_	640
-			Leu	645			•		650					655	-
	_	_	Gly 660	_	_	-	_	665		_		_	670	_	
		675	Ile Ile		-	_	680					685			
	690	_	Ser			695			_	_	700			_	
705	Deu	Mec	361	GIII	710	БУЗ	пеп	GIU	Lys	715	FIIC	GIY	GIII	Jer	720
Tla	Dha	Tla	λla	Car		Dhe	Mot	ጥኩሎ	Gln.		Glaz	Tla	Dro	Pro	
			Ala	725	Thr				730	Gly				735	Ser
Thr	Asn	Pro	Ala 740	725 Ser	Thr Leu	Leu	Lys	Glu 745	730 Ala	Gly	His	Val	Ile 750	735 Ser	Ser Cys
Thr Gly	Asn Tyr	Pro Glu 755	Ala 740 Asp	725 Ser Lys	Thr Leu Thr	Leu Glu	Lys Trp 760	Glu 745 Gly	730 Ala Lys	Gly Ile Glu	His Ile	Val Gly 765	Ile 750 Trp	735 Ser Ile	Ser Cys Tyr
Thr Gly	Asn Tyr Ser 770	Pro Glu 755 Val	Ala 740 Asp Thr	725 Ser Lys Glu	Thr Leu Thr Asp	Leu Glu Ile 775	Lys Trp 760 Leu	Glu 745 Gly Thr	730 Ala Lys Gly	Gly Ile Glu Phe	His Ile Lys 780	Val Gly 765 Met	Ile 750 Trp His	735 Ser Ile Ala	Ser Cys Tyr Arg
Thr Gly	Asn Tyr Ser 770	Pro Glu 755 Val	Ala 740 Asp	725 Ser Lys Glu	Thr Leu Thr Asp	Leu Glu Ile 775	Lys Trp 760 Leu	Glu 745 Gly Thr	730 Ala Lys Gly	Gly Ile Glu Phe	His Ile Lys 780	Val Gly 765 Met	Ile 750 Trp His	735 Ser Ile Ala	Ser Cys Tyr Arg
Thr Gly Gly 785 Ser	Asn Tyr Ser 770 Trp	Pro Glu 755 Val Gln Pro	Ala 740 Asp Thr Ser	725 Ser Lys Glu Ile Asn 805	Thr Leu Thr Asp Tyr 790 Leu	Leu Glu Ile 775 Cys Ser	Lys Trp 760 Leu Met	Glu 745 Gly Thr Pro	730 Ala Lys Gly Pro Leu 810	Gly Ile Glu Phe Arg 795 Asn	His Ile Lys 780 Pro	Val Gly 765 Met Cys Val	Ile 750 Trp His Phe Leu	735 Ser Ile Ala Lys Arg 815	Ser Cys Tyr Arg Gly 800 Trp
Thr Gly Gly 785 Ser	Asn Tyr Ser 770 Trp Ala Leu	Pro Glu 755 Val Gln Pro Gly	Ala 740 Asp Thr Ser Ile Ser 820	725 Ser Lys Glu Ile Asn 805 Val	Thr Leu Thr Asp Tyr 790 Leu Glu	Leu Glu Ile 775 Cys Ser Ile	Lys Trp 760 Leu Met Asp	Glu 745 Gly Thr Pro Arg Leu 825	730 Ala Lys Gly Pro Leu 810 Ser	Gly Ile Glu Phe Arg 795 Asn	His Ile Lys 780 Pro Gln His	Val Gly 765 Met Cys Val	Ile 750 Trp His Phe Leu Pro 830	735 Ser Ile Ala Lys Arg 815 Ile	Ser Cys Tyr Arg Gly 800 Trp
Thr Gly Gly 785 Ser Ala	Asn Tyr Ser 770 Trp Ala Leu Gly	Pro Glu 755 Val Gln Pro Gly Tyr 835	Ala 740 Asp Thr Ser Ile Ser 820 Asn	725 Ser Lys Glu Ile Asn 805 Val	Thr Leu Thr Asp Tyr 790 Leu Glu Arg	Leu Glu Ile 775 Cys Ser Ile Leu	Lys Trp 760 Leu Met Asp Leu Lys 840	Glu 745 Gly Thr Pro Arg Leu 825 Leu	730 Ala Lys Gly Pro Leu 810 Ser Leu	Gly Ile Glu Phe Arg 795 Asn Arg	His Ile Lys 780 Pro Gln His	Val Gly 765 Met Cys Val Cys Leu 845	Ile 750 Trp His Phe Leu Pro 830 Ala	735 Ser Ile Ala Lys Arg 815 Ile	Ser Cys Tyr Arg Gly 800 Trp Trp
Thr Gly Gly 785 Ser Ala Tyr Asn	Asn Tyr Ser 770 Trp Ala Leu Gly Thr	Pro Glu 755 Val Gln Pro Gly Tyr 835 Ile	Ala 740 Asp Thr Ser Ile Ser 820 Asn	725 Ser Lys Glu Ile Asn 805 Val Gly	Thr Leu Thr Asp Tyr 790 Leu Glu Arg	Leu Glu Ile 775 Cys Ser Ile Leu Ile 855	Lys Trp 760 Leu Met Asp Leu Lys 840 Thr	Glu 745 Gly Thr Pro Arg Leu 825 Leu Ser	730 Ala Lys Gly Pro Leu 810 Ser Leu Ile	Gly Ile Glu Phe Arg 795 Asn Arg Glu Pro	His Ile Lys 780 Pro Gln His Arg Leu 860	Val Gly 765 Met Cys Val Cys Leu 845 Ile	Ile 750 Trp His Phe Leu Pro 830 Ala	735 Ser Ile Ala Lys Arg 815 Ile Tyr	Ser Cys Tyr Arg Gly 800 Trp Trp Ile Cys
Thr Gly Gly 785 Ser Ala Tyr Asn Val 865	Asn Tyr Ser 770 Trp Ala Leu Gly Thr 850 Leu	Pro Glu 755 Val Gln Pro Gly Tyr 835 Ile	Ala 740 Asp Thr Ser Ile Ser 820 Asn Val	725 Ser Lys Glu Ile Asn 805 Val Gly Tyr	Thr Leu Thr Asp Tyr 790 Leu Glu Arg Pro Cys 870	Leu Glu Ile 775 Cys Ser Ile Leu Ile 855 Leu	Lys Trp 760 Leu Met Asp Leu Lys 840 Thr	Glu 745 Gly Thr Pro Arg Leu 825 Leu Ser	730 Ala Lys Gly Pro Leu 810 Ser Leu Ile Asn	Gly Ile Glu Phe Arg 795 Asn Arg Glu Pro Lys 875	His Ile Lys 780 Pro Gln His Arg Leu 860 Phe	Val Gly 765 Met Cys Val Cys Leu 845 Ile	Ile 750 Trp His Phe Leu Pro 830 Ala Ala Ile	735 Ser Ile Ala Lys Arg 815 Ile Tyr Tyr	Ser Cys Tyr Arg Gly 800 Trp Trp Ile Cys Glu 880
Thr Gly Gly 785 Ser Ala Tyr Asn Val 865 Ile	Asn Tyr Ser 770 Trp Ala Leu Gly Thr 850 Leu Ser	Pro Glu 755 Val Gln Pro Gly Tyr 835 Ile Pro Asn	Ala 740 Asp Thr Ser Ile Ser 820 Asn Val Ala Tyr	725 Ser Lys Glu Ile Asn 805 Val Gly Tyr Ile Ala 885	Thr Leu Thr Asp Tyr 790 Leu Glu Arg Pro Cys 870 Gly	Leu Glu Ile 775 Cys Ser Ile Leu Ile 855 Leu Met	Lys Trp 760 Leu Met Asp Leu Lys 840 Thr Leu Phe	Glu 745 Gly Thr Pro Arg Leu 825 Leu Ser Thr	730 Ala Lys Gly Pro Leu 810 Ser Leu Ile Asn Ile 890	Gly Ile Glu Phe Arg 795 Asn Arg Glu Pro Lys 875 Leu	His Ile Lys 780 Pro Gln His Arg Leu 860 Phe	Val Gly 765 Met Cys Val Cys Leu 845 Ile Ile	Ile 750 Trp His Phe Leu Pro 830 Ala Ala Ile	735 Ser Ile Ala Lys Arg 815 Ile Tyr Tyr Pro Ser 895	Ser Cys Tyr Arg Gly 800 Trp Trp Cys Glu 880 Ile
Thr Gly Gly 785 Ser Ala Tyr Asn Val 865 Ile	Asn Tyr Ser 770 Trp Ala Leu Gly Thr 850 Leu Ser Ala	Pro Glu 755 Val Gln Pro Gly Tyr 835 Ile Pro Asn	Ala 740 Asp Thr Ser Ile Ser 820 Asn Val Ala Tyr Gly 900	725 Ser Lys Glu Ile Asn 805 Val Gly Tyr Ile Ala 885 Ile	Thr Leu Thr Asp Tyr 790 Leu Glu Arg Pro Cys 870 Gly Leu	Leu Glu Ile 775 Cys Ser Ile Leu Ile 855 Leu Met Glu	Lys Trp 760 Leu Met Asp Leu Lys 840 Thr Leu Phe	Glu 745 Gly Thr Pro Arg Leu 825 Leu Ser Thr Phe	730 Ala Lys Gly Pro Leu 810 Ser Leu Ile Asn Ile 890 Trp	Gly Ile Glu Phe Arg 795 Asn Arg Glu Pro Lys 875 Leu Ser	His Ile Lys 780 Pro Gln His Arg Leu 860 Phe Leu Gly	Val Gly 765 Met Cys Val Cys Leu 845 Ile Ile Phe	Ile 750 Trp His Phe Leu Pro 830 Ala Ala Ile Ala Gly 910	735 Ser Ile Ala Lys Arg 815 Ile Tyr Tyr Pro Ser 895 Ile	Cys Tyr Arg Gly 800 Trp Trp Cys Glu 880 Ile Glu
Gly Gly 785 Ser Ala Tyr Asn Val 865 Ile Phe Asp	Asn Tyr Ser 770 Trp Ala Leu Gly Thr 850 Leu Ser Ala Trp	Pro Glu 755 Val Gln Pro Gly Tyr 835 Ile Pro Asn Thr	Ala 740 Asp Thr Ser Ile Ser 820 Asn Val Ala Tyr Gly 900 Arg	725 Ser Lys Glu Ile Asn 805 Val Gly Tyr Ile Ala 885 Ile Asn	Thr Leu Thr Asp Tyr 790 Leu Glu Arg Pro Cys 870 Gly Leu Glu	Leu Glu Ile 775 Cys Ser Ile Leu Ile 855 Leu Met Glu Gln	Lys Trp 760 Leu Met Asp Leu Lys 840 Thr Leu Phe Leu Phe	Glu 745 Gly Thr Pro Arg Leu 825 Leu Ser Thr Phe Arg 905	730 Ala Lys Gly Pro Leu 810 Ser Leu Ile Asn Ile 890 Trp	Gly Ile Glu Phe Arg 795 Asn Arg Glu Pro Lys 875 Leu Ser Ile	His Ile Lys 780 Pro Gln His Arg Leu 860 Phe Leu Gly	Val Gly 765 Met Cys Val Cys Leu 845 Ile Ile Phe Val Gly 925	Ile 750 Trp His Phe Leu Pro 830 Ala Ala Ile Ala Gly 910 Thr	735 Ser Ile Ala Lys Arg 815 Ile Tyr Tyr Pro Ser 895 Ile Ser	Cys Tyr Arg Gly 800 Trp Trp Ile Cys Glu 880 Ile Glu Ala

WO 00/09706 PCT/US99/18760

- 110 -

```
935
    930
Asp Thr Asn Phe Thr Val Thr Ser Lys Ala Ser Asp Glu Asp Gly Asp
                 950
                                        955
Phe Ala Glu Leu Tyr Val Phe Lys Trp Thr Ser Leu Leu Ile Pro Pro
                                    970
                965
Thr Thr Val Leu Val Ile Asn Leu Val Gly Met Val Ala Gly Ile Ser
                                                    990
            980
                                985
Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys
                                                1005
                            1000
Leu Phe Phe Ser Ile Trp Val Ile Leu His Leu Tyr Pro Phe Leu Lys
                        1015
Gly Leu Met Gly Arg Gln Asn Arg Thr Pro Thr Ile Val Ile Val Trp
                                        1035
1025
                     1030
Ser Ile Leu Leu Ala Ser Ile Phe Ser Leu Leu Trp Val Lys Ile Asp
                                    1050
Pro Phe Ile Ser Pro Thr Gln Lys Ala Ala Ala Leu Gly Gln Cys Gly
                                 1065
                                                     1070
Val Asn Cys
        1075
       <210> 55
       <211> 25
       <212> DNA
       <213> Zea mays
       <400> 55
atggcggcca acaaggggat ggtgg
25
       <210> 56
       <211> 25
       <212> DNA
       <213> Zea mays
       <400> 56
 teageagttg acgccacatt gcccc
25
       <210> 57
       <211> 3704
       <212> DNA
       <213> Zea mays
       <220>
       <221> CDS
       <222> (272)...(3497)
       <400> 57
                                                                       60
 gtcgacccac gcttccggtc ggttccgcgt cccttttccc ctccccctc cgtcgccgcc
 tegagegage tecaceaett geteetgege gaggtgaaca etgggttagg gecaetgeea
                                                                       120
                                                                       180
 cegetggget geetetgett etgeetetee egeeagegeg egageeeggg ggegattegg
 cgccggcacg cgggaggga agccgaggaa tgcggtgagt cggcgggggt ccggcgtttg
                                                                       240
                                                                       292
 tgaactcgtg gagggctcgg attggtgcgc c atg gac ggc ggc gac gcc acg
                                    Met Asp Gly Gly Asp Ala Thr
 aat tog ggg aag cat gtg gcc ggg cag gtg tgc cag atc tgc ggc gac
                                                                       340
 Asn Ser Gly Lys His Val Ala Gly Gln Val Cys Gln Ile Cys Gly Asp
```

- 111 -

		10					15					20				
ggc g Gly V	_					_						-		-		388
tgc g Cys G 40																436
ggc a Gly T		_		_	_	_	_	_		_		_	_			484
ggg a Gly S																532
gat g Asp V	-	_	_					_				_	_	_	_	580
caa a Gln L																628
agt g Ser A 120																676
aag t Lys T		_	_					_				_				724
cat a His S																772
atg t Met S								Arg								820
gta a Val A																868
aat g Asn V 200	gtt Val	gca Ala	tgg Trp	aaa Lys	gag Glu 205	agg Arg	gtg Val	gat Asp	gga Gly	tgg Trp 210	aaa Lys	atg Met	aag Lys	gat Asp	aaa Lys 215	916
ggt g Gly A																964
cgt g Arg G	gga Gly	gtt Val	gct Ala 235	gat Asp	att Ile	gat Asp	gct Ala	tct Ser 240	Thr	gat Asp	tat Tyr	aac Asn	atg Met 245	Glu	gat Asp	1012

- 112 -

_		_		_	gaa Glu							_				106	50
					ata Ile											110	80
					ata Ile 285											11,	56
					ctg Leu											120	04
					att Ile											12!	52
					ctt Leu											13	00
					tta Leu											13	48
					ect Pro 365											13:	96
					tat Tyr											14	44
					atg Me t											14	92
					tgg Trp											15	40
cct Pro	ang Xaa 425	gcc Ala	ccg Pro	gaa Glu	tgg Trp	tac Tyr 430	ttt Phe	gct Ala	cag Gln	aaa Lys	att Ile 435	gat Asp	tac Tyr	ttg Leu	aaa Lys	15	88
gac Asp 440	Lys	gtt Val	caa Gln	acc Thr	tca Ser 445	ttt Phe	gtg Val	aaa Lys	gaa Glu	cgc Arg 450	Arg	gcc Ala	atg Met	aag Lys	aga Arg 455	16	36
gaa Glu	tat Tyr	gaa Glu	gaa Glu	ttc Phe 460	aaa Lys	gtt Val	cgt Arg	atc Ile	aat Asn 465	Gly	ctt Leu	gta Val	gcc	aag Lys 470	Ala	16	84
caa Gln	aaa Lys	gtt Val	ccc	gag Glu	gag Glu	gga Gly	tgg Trp	ato	atg Met	caa Gln	gat Asp	ggt Gly	aca Thr	cct Pro	tgg Trp	17	32

- 113 -

								- 11	J -							
			475					480		•			485			
cct Pro	gjå aaa	aac Asn 490	aat Asn	act Thr	agg Arg	gac Asp	cat His 495	cct Pro	gga Gly	atg Met	att Ile	cag Gln 500	gtt Val	ttc Phe	ctg Leu	1780
ggt Gly	cac His 505	agt Ser	gga Gly	GJÀ aaa	ctt Leu	gac Asp 510	gtt Val	gaa Glu	ggc Gly	aat Asn	gaa Glu 515	ctt Leu	cct Pro	cgt Arg	ttg Leu	1828
gtt Val 520	tat Tyr	gtg Val	tct Ser	cgt Arg	gaa Glu 525	aaa Lys	cgt Arg	cct Pro	gga Gly	ttc Phe 530	caa Gln	cat His	cac His	aag Lys	aag Lys 535	1876
gct Ala	ggt Gly	gcc Ala	atg Met	aat Asn 540	gca Ala	ctt Leu	gtt Val	cgt Arg	gta Val 545	tca Ser	gct Ala	gtc Val	ctt Leu	act Thr 550	aat Asn	1924
gjà aaa	caa Gln	tac Tyr	atg Met 555	ttg Leu	aat Asn	ctt Leu	gat Asp	tgt Cys 560	gac Asp	cac His	tac Tyr	atc Ile	aat Asn 565	aat Asn	agc Ser	1972
aag Lys	gct Ala	ctt Leu 570	cga Arg	gaa Glu	gct Ala	atg Met	tgc Cys 575	ttc Phe	ctt Leu	atg Met	gac Asp	cca Pro 580	aac Asn	cta Leu	gga Gly	2020
agg Arg	aat Asn 585	gtc Val	tgt Cys	tat Tyr	gtc Val	caa Gln 590	ttt Phe	cct Pro	cag Gln	agg Arg	ttt Phe 595	gat Asp	ggt Gly	att Ile	gat Asp	2068
agg Arg 600	Asn	gac Asp	cga Arg	tat Tyr	gca Ala 605	aac Asn	agg Arg	aac Asn	act Thr	gtg Val 610	Phe	ttc Phe	gat Asp	att Ile	aac Asn 615	2116
ttg Leu	aga Arg	ggt	ctt Leu	gac Asp 620		att Ile	caa Gln	gly	cca Pro 625	Val	tat Tyr	gtg Val	gga Gly	act Thr 630	ggt Gly	2164
tgt Cys	gtg Val	ttt Phe	аас Азп 635	Arg	acg Thr	gcc	Leu	tat Tyr 640	Gly	tat Tyr	Glu	Pro	Pro 645	Val	aag Lys	2212
aaa Lys	aaa Lys	aag Lys 650	Pro	ggc Gly	tto Phe	ttc Phe	tct Ser 655	Ser	ctt Leu	tgt Cys	ggg Gly	gga Gly 660	Arg	aaa Lys	aag Lys	2260
acg Thr	tca Ser 665	Lys	tct Ser	aag Lys	g aag Lys	ago Ser 670	Ser	gaa Glu	aag Lys	r aag Lys	aag Lys 675	Ser	cat His	aga Arg	cac His	2308
gca Ala 680	a Asp	agt Sei	tct Sei	gta va:	a cca l Pro 685	val	ttt Phe	aat Asr	cto Lev	gaa Glu 690	ı Asç	ata Ile	gag Glu	ggaa a Glu	ggg Gly 695	2356
att Ile	gaa Glu	a ggt u Gly	t tot y Sei	caq c Gli	n Phe	gat Asp	gat	gag Glu	g aaa 1 Lys 70:	s Se	g cto	g att	atg Met	tct Sei 710	caa Gln	2404

- 114 -

atg Met	agc Ser	ttg Leu	gag Glu 715	aag Lys	aga Arg	ttt Phe	ggc Gly	cag Gln 720	tcc Ser	agt Ser	gtt Val	ttt Phe	gta Val 725	gcc Ala	tct Ser	2452	
act Thr	ctg Leu	atg Met 730	gaa Glu	tat Tyr	ggt Gly	ggt Gly	gtt Val 735	cca Pro	caa Gln	tct Ser	gca Ala	act Thr 740	cca Pro	gag Glu	tct Ser	2500	j
														gac Asp		2548	ļ
act Thr 760	gac Asp	tgg Trp	gga Gly	act Thr	gag Glu 765	att Ile	GJÀ aaa	tgg Trp	atc Ile	tat Tyr 770	ggt Gly	tct Ser	gtt Val	aca Thr	gaa Glu 775	2596	;
gac Asp	att Ile	ctc Leu	acc Thr	gga Gly 780	ttc Phe	aag Lys	atg Met	cat His	gct Ala 785	cga Arg	ggc Gly	tgg Trp	cga Arg	tca Ser 790	atc Ile	2644	ļ
tac Tyr	tgc Cys	atg Met	cct Pro 795	aag Lys	cga Arg	cca Pro	gct Ala	ttc Phe 800	aag Lys	gga Gly	tct Ser	gct Ala	cct Pro 805	atc Ile	aac Asn	2692	2
ctt Leu	tcg Ser	gat Asp 810	Arg	ttg Leu	aat Asn	caa Gln	gtg Val 815	ctt Leu	cgg Arg	tgg Trp	gct Ala	ctt Leu 820	ggt Gly	tcc Ser	att Ile	2740)
gaa Glu	att Ile 825	ctt Leu	ttc Phe	agc Ser	agg Arg	cat His 830	tgt Cys	ccc Pro	ata Ile	tgg Trp	tat Tyr 835	ggc	tat Tyr	gga Gly	ggc Gly	2788	3
cgg Arg 840	Leu	aaa Lys	ttc Phe	ctg Leu	gag Glu 845	aga Arg	ttt Phe	gct Ala	tat Tyr	atc Ile 850	Asn	aca Thr	aca Thr	att Ile	tat Tyr 855	283	5
cca Pro	ctc Leu	aca Thr	tca Ser	atc Ile 860	Pro	ctc Leu	ctc Leu	ctg Leu	tac Tyr 865	Cys	ata Ile	ttg Leu	cca Pro	gca Ala 870	gtt Val	288	4
tgt Cys	ctt Leu	ctc Leu	act Thr 875	Gly	aag Lys	ttc Phe	atc Ile	atc Ile 880	Pro	aag Lys	att Ile	agt Ser	aac Asn 885	cta Leu	gag Glu	293	2
agt Ser	gtt Val	tgg Trp	Phe	ata Ile	tcg Ser	ctc Leu	ttt Phe 895	Ile	tca Ser	atc Ile	ttt Phe	gcc Ala	Thr	ggt Gly	atc Ile	298	0
ctt	gag Glu 905	Met	agg : Arg	tgg Trp	agt Ser	ggt Gly 910	Val	ggc	att Ile	gat Asp	gaa Glu 915	Trp	tgg Trp	agg Arg	aac Asn	302	8
gag Glu 920	Gln	tto Phe	tgg Trp	gto Val	att Ile	Gly	ggt Gly	att lle	tct Ser	gcg Ala 930	a His	tta Lev	ttt Phe	gcc Ala	gtc Val 935	307	6
tto Phe	cag	ggt Gly	cto Lev	cto Lev	ı aagı ı Lys	gtg Val	r ctt . Lei	gct Ala	ggt Gly	ato	gac Asp	acg Thi	g ago	tto Phe	act Thr	312	4

- 115 -

	- +	13 -	
940		945	950
gtc acc tct aag gcc Val Thr Ser Lys Ala 955			Leu Tyr
atg ttc aag tgg aca Met Phe Lys Trp Thr 970			_
atc aac ctg gtc ggc Ile Asn Leu Val Gly 985			
ggt tac cag tca tgg Gly Tyr Gln Ser Trp 1000			
tgg gtg att gtc cac Trp Val Ile Val His 1020	Leu Tyr Pro Phe		
cag aac cgc acg ccg Gln Asn Arg Thr Pro 1035		Val Trp Ala Ile Leu	Leu Ala
tcg atc ttt tcc ctg Ser Ile Phe Ser Leu 1050			
gtc act ggc cct gat Val Thr Gly Pro Asp 1065			gatga 3507
gctgaagata gttaaagag gctcttttta tagtatggt acctccgctg gtctttatc aaaaaaaggg cggccgc	a ggaacttggt cg	ggagacgt taattacata	tgctatatgt 3627
<210> 58 <211> 1076 <212> PRT <213> Zea mays	3		·
<pre><400> 58 Met Asp Gly Gly Asp</pre>	Ala Thr Asn Ser		_
1 5 Val Cys Gln Ile Cys 20	Gly Asp Gly Val	Gly Thr Ala Ala Asp	15 Gly Asp
Leu Phe Thr Ala Cys			Pro Cys
Tyr Glu Tyr Glu Arg		==	Cys Lys
Thr Lys Tyr Lys Arg	His Lys Gly Ser 70	Pro Pro Val His Gly 75	Glu Glu 80
Asn Glu Asp Val Asp 85	Ala Asp Asp Val		Gln Ala 95
Ser Gly Asn Gln Asp	_	90	

- 116 -

			100					105					110		
Trp	Arg	Thr 115	Asn	Ser	Arg	Gly	Ser 120	qaA	Ile	Gly	Leu	Ala 125	Lys	Tyr	Asp
Ser	Gly 130	Glu	Ile	Gly	His	Gly 135	Lys	Tyr	qaA	Ser	Gly 140	Glu	Ile	Pro	Arg
145	_				150					Ile 155					160
_				165					170	Val				175	
_	-		180			-		185		Ser			190		_
		195	_				200			Trp		205			
	210					215	·			Pro	220				
Ser 225	Ile	Ala	Pro	Ser	Glu 230	Gly	Arg	Gly	Val	Ala 235	Asp	Ile	Asp	Ala	Ser 240
	Asp	Tyr	Asn	Met 245		Asp	Ala	Leu	Leu 250	Asn	Asp	Glu	Thr	Arg 255	
Pro	Leu	Ser	Arg 260	Lys	Val	Pro	Ile	Pro 265	Ser	Ser	Arg	Ile	Asn 270	Pro	Tyr
Arg	Met	Val 275	Ile	Val	Leu	Arg	Leu 280	Ala	Val	Leu	Cys	Ile 285	Phe	Leu	Arg
•	290					295				Tyr	300		_		•
Ser 305	Val	Ile	Cys	Glu	Ile 310	Trp	Phe	Ala	Leu	Ser 315	Trp	Ile	Leu	Asp	Gln 320
	Pro	Lys	Trp	Ser 325		Ile	Asn	Arg	Glu 330	Thr	Tyr	Leu	Asp	Arg 335	
			340					345		Ser			350		
_		355					360			Lys		365			
	370					375				Val	380				
Lys 385	Val	Ser	Cys	Tyr	Val 390	Ser	Asp	Asp	Gly	Ala 395	Ala	Met	Leu	Thr	Phe 400
	Ala	Leu	Ser	Glu 405		Ser	Glu	Phe	Ala 410	Arg	Lys	Trp	Val	Pro 415	Phe
-	_	_	420					425		Pro			430		
		435					440					445			Lys
	450	_			_	455		_			460				Ile
Asn 465	Gly	Leu	Val	Ala	Lys 470		Gln	Lys	Val	Pro 475	Glu	Glu	GIÀ	Trp	Ile 480
	Gln	Asp	Gly	Thr 485	Pro		Pro	Gly	Asn 490	Asn	Thr	Arg	Asp	His 495	Pro
Gly	Met	Ile	Gln 500	Val	Phe	Leu	Gly	His 505		Gly	Gly	Leu	Asp 510		Glu
_		515					520					525			Pro
_	530					535					540				Arg
Val 545		Ala	Val	Leu	Thr 550		Gly	Gln	Tyr	Met 555		Asn	Leu	qaA	Cys
		Tyr	Ile	Asn			Lys	Ala	Leu			Ala	Met	Cys	Phe

WO 00/09706 PCT/US99/18760

- 117 -

Leu Met Asp Pro Asn Leu Gly Arg Asn Val Cys Tyr Val Gln Phe Pro Gln Arg Phe Asp Gly Ile Asp Arg Asn Asp Arg Tyr Ala Asn Arg Asn Thr Val Phe Phe Asp Ile Asn Leu Arg Gly Leu Asp Gly Ile Gln Gly Pro Val Tyr Val Gly Thr Gly Cys Val Phe Asn Arg Thr Ala Leu Tyr Gly Tyr Glu Pro Pro Val Lys Lys Lys Pro Gly Phe Phe Ser Ser Leu Cys Gly Gly Arg Lys Lys Thr Ser Lys Ser Lys Ser Ser Glu Lys Lys Lys Ser His Arg His Ala Asp Ser Ser Val Pro Val Phe Asn Leu Glu Asp Ile Glu Glu Gly Ile Glu Gly Ser Gln Phe Asp Asp Glu Lys Ser Leu Ile Met Ser Gln Met Ser Leu Glu Lys Arg Phe Gly Gln Ser Ser Val Phe Val Ala Ser Thr Leu Met Glu Tyr Gly Gly Val Pro Gln Ser Ala Thr Pro Glu Ser Leu Leu Lys Glu Ala Ile His Val Ile Ser Cys Gly Tyr Glu Asp Lys Thr Asp Trp Gly Thr Glu Ile Gly Trp Ile Tyr Gly Ser Val Thr Glu Asp Ile Leu Thr Gly Phe Lys Met His Ala Arg Gly Trp Arg Ser Ile Tyr Cys Met Pro Lys Arg Pro Ala Phe Lys Gly Ser Ala Pro Ile Asn Leu Ser Asp Arg Leu Asn Gln Val Leu Arg Trp Ala Leu Gly Ser Ile Glu Ile Leu Phe Ser Arg His Cys Pro Ile Trp Tyr Gly Tyr Gly Gly Arg Leu Lys Phe Leu Glu Arg Phe Ala Tyr Ile Asn Thr Thr Ile Tyr Pro Leu Thr Ser Ile Pro Leu Leu Tyr Cys Ile Leu Pro Ala Val Cys Leu Leu Thr Gly Lys Phe Ile Ile Pro Lys Ile Ser Asn Leu Glu Ser Val Trp Phe Ile Ser Leu Phe Ile Ser Ile Phe Ala Thr Gly Ile Leu Glu Met Arg Trp Ser Gly Val Gly Ile Asp Glu Trp Trp Arg Asn Glu Gln Phe Trp Val Ile Gly Gly Ile Ser Ala His Leu Phe Ala Val Phe Gln Gly Leu Leu Lys Val Leu Ala Gly Ile Asp Thr Ser Phe Thr Val Thr Ser Lys Ala Thr Asp Glu Glu Gly Asp Phe Ala Glu Leu Tyr Met Phe Lys Trp Thr Thr Leu Leu Ile Pro Pro Thr Thr Ile Leu Ile Ile Asn Leu Val Gly Val Val Ala Gly Ile Ser Tyr Ala Ile Asn Ser Gly Tyr Gln Ser Trp Gly Pro Leu Phe Gly Lys Leu Phe Phe Ala Phe Trp Val Ile Val His Leu Tyr Pro Phe Leu Lys Gly Leu Met Gly Lys Gln Asn Arg Thr Pro Thr Ile Val Val WO 00/09706 PCT/US99/18760

- 118 -

1035 1025 1030 1040 Val Trp Ala Ile Leu Leu Ala Ser Ile Phe Ser Leu Met Trp Val Arg 1045 1050 Ile Asp Pro Phe Thr Thr Arg Val Thr Gly Pro Asp Ile Ala Lys Cys 1060 1065 Gly Ile Asn Cys 1075 <210> 59 <211> 25 <212> DNA <213> Zea mays <400> 59 atggacggcg gcgacgccac gaatt 25 <210> 60 <211> 25 <212> DNA <213> Zea mays <400> 60 ctagcagttg atgccacatt tcgcg 25

119

WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7: WO 00/09706 (11) International Publication Number: **A3** C12N 15/54, 9/10, 5/10, 15/82 (43) International Publication Date: 24 February 2000 (24.02.00) PCT/US99/18760 (21) International Application Number: (81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, (22) International Filing Date: 16 August 1999 (16.08.99) GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, (30) Priority Data: SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, 17 August 1998 (17.08.98) 60/096,822 US ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI (71) Applicant (for all designated States except US): PIONEER HI-BRED INTERNATIONAL, INC. [US/US]; 800 Capital patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, Square, 400 Locust Street, Des Moines, IA 50309 (US). NE, SN, TD, TG). (72) Inventors; and (75) Inventors/Applicants (for US only): DHUGGA, Kanwarpal, Published S. [US/US]; 8320 Barnham Drive, Johnston, IA 50131 With international search report. (US). HELENTJARIS, Timothy, G. [US/US]; 2960 N.W. 73rd Lane, Ankeny, IA 50021 (US). BOWEN, Benjamin, (88) Date of publication of the international search report: A. [GB/US]; 7027 Buckingham Boulevard, Berkeley, CA 16 November 2000 (16.11.00) 94705 (US). WANG, Xun [CN/US]; 12524 Caminito Vista Soledad, San Diego, CA 92130 (US). (74) Agents: BLAIR, Debra, L. et al.; 7100 N.W. 62nd Avenue, Darwin Building, Johnston, IA 50131-1000 (US).

(54) Title: MAIZE CELLULOSE SYNTHASES AND USES THEREOF

(57) Abstract

The invention provides isolated cellulose synthase nucleic acids and their encoded proteins. The present invention provides methods and compositions relating to altering cellulose synthase concentration and/or composition of plants. The invention further provides recombinant expression cassettes, host cells, and transgenic plants.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM Armenia FI Finland LT Lithuania SK Slovakia AT Austria FR France LU Luxembourg SN Senegal AU Australia GA Gabon LV Latvia SZ Swaziland AZ Azerbaijan GB United Kingdom MC Monaco TD Chad BA Bosnia and Herzegovina GE Georgia MD Republic of Moldova TG Togo BB Barbados GH Ghana MG Madagascar TJ Tajikistan BE Belgium GN Guinea MK The former Yugoslav TM Turkmenistan BF Burkina Faso GR Greece Republic of Macedonia TR Turkey BG Bulgaria HU Hungary ML Mali TT Trinidad and Tobago BJ Benin IE Ireland MN Mongolia UA Ulraine BR Brazil II Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CM Cameroon Republic of Korea PL Poland CN Cameroon Republic of Korea PL Poland CC Czecch Republic LC Saint Lucia RU Russian Federation DK Denmark LK Sri Lanka SE Sweden EE Bstonia LR Liberia SG Singapore	AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia	
AU Australia GA Gabon LV Latvia SZ Swaziland AZ Azerbaijan GB United Kingdom MC Monaco TD Chad BA Bosnia and Herzegovima GE Georgia MD Republic of Moldova TG Togo BB Barbados GH Ghana MG Madagascar TJ Tajikistan BE Belgium GN Guinea MK The former Yugoslav TM Turkmenistan BF Burkina Faso GR Greece Republic of Macedonia TR Turkey BG Bulgaria HU Hungary ML Mali TT Trinidad and Tobago BJ Benin IE Ireland MN Mongolia UA Ukraine BR Brazil IIL Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CC Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liebenstein SD Sudan DK Demmark LK Sri Lanka SE Sweden	AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia	
AZ Azerbaijan GB United Kingdom MC Monaco TD Chad BA Bosnia and Herzegovina GE Georgia MD Republic of Moldova TG Togo BB Barbados GH Ghana MG Madagascar TJ Tajikistan BE Belgium GN Guinea MK The former Yugoslav TM Turkmenistan BF Burkina Faso GR Greece Republic of Macedonia TR Turkey BG Bulgaria HU Hungary ML Mali TT Trinidad and Tobago BJ Benin IE Ireland MN Mongolia UA Ukraine BR Brazil II. Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PL Poland CC Czech Republic LC Saint Lucia RU Russian Federation DE Germany L1 Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	ΑT	Austria	FR	France	LU	Luxembourg	SN	Senegal	
BA Bosnia and Herzegovina GE Georgia MD Republic of Moldova TG Togo BB Barbados GH Ghana MG Madagascar TJ Tajikistan BE Belgium GN Guinea MK The former Yugoslav TM Turkmenistan BF Burkina Faso GR Greece Republic of Macedonia TR Turkey BG Bulgaria HU Hungary ML Mali TT Trinidad and Tobago BJ Benin IE Ireland MN Mongolia UA Ukraine BR Brazil II. Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CCH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CN China KR Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CC Cermany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	ΑÜ	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland	
BB Barbados GH Ghana MG Madagascar TJ Tajikistan BE Belgium GN Guinea MK The former Yugoslav TM Turkmenistan BF Burkina Faso GR Greece Republic of Macedonia TR Turkey BG Bulgaria HU Hungary ML Mali TT Trinidad and Tobago BJ Benin IE Ireland MN Mongolia UA Ukraine BR Brazil II. Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CN Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PL Potrugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic CZ Czech Republic CE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	AZ	Azerbaijan	GB	United Kingdom	MC	Моласо	TD	Chad	
BE Belgium GN Guinea MK The former Yugoslav TM Turkmenistan BF Burkina Faso GR Greece Republic of Macedonia TR Turkey BG Bulgaria HU Hungary ML Mali TT Trinidad and Tobago BJ Benin IE Ireland MN Mongolia UA Ukraine BR Brazil II. Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo	
BF Burkina Faso GR Greece Republic of Macedonia TR Turkey BG Bulgaria HU Hungary ML Mali TT Trinidad and Tobago BJ Benin IE Ireland MN Mongolia UA Ukraine BR Brazil II. Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DK Denmark LK Sri Lanka SE Sweden	BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan	
BG Bulgaria HU Hungary ML Mali TT Trinidad and Tobago BJ Benin IE Ireland MN Mongolia UA Ukraine BR Brazil II. Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekstam CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NI. Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan	
BI Benin IE Ireland MIN Mongolia UA Ukraime BR Brazil II. Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	BF	Burkina Faso	GR	Greece		Republic of Macedonia	TR	Turkey	
BR Brazil II. Israel MR Mauritania UG Uganda BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago	
BY Belarus IS Iceland MW Malawi US United States of America CA Canada IT Italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic CZ Czech Republic CZ Czech Republic CD Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	BJ	Benin	ΙE	Ireland	MN	Mongolia	UA	Ukraine	
CA Canada IT italy MX Mexico UZ Uzbekistan CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	BR	Brazil	IL.	Israel	MR	Mauritania	UG	Uganda	
CF Central African Republic JP Japan NE Niger VN Viet Nam CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America	
CG Congo KE Kenya NL Netherlands YU Yugoslavia CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	CA	Canada	ΙT	Italy	MX	Mexi∞	UZ	Uzbekistan	
CH Switzerland KG Kyrgyzstan NO Norway ZW Zimbabwe CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam	
CI Côte d'Ivoire KP Democratic People's NZ New Zealand CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia	
CM Cameroon Republic of Korea PL Poland CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	CH	Switzerland	KG	Kyrgyzstan	NO	Norway	zw	Zimbabwe	
CN China KR Republic of Korea PT Portugal CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		•	
CU Cuba KZ Kazakstan RO Romania CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	CM	Cameroon		Republic of Korea	PL	Poland			
CZ Czech Republic LC Saint Lucia RU Russian Federation DE Germany LI Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	CN	China	KR	Republic of Korea	PT	Portugal			
DE Germany Li Liechtenstein SD Sudan DK Denmark LK Sri Lanka SE Sweden	CU	Cuba	KZ	Kazakstan	RO	Romania			
DK Denmark LK Sri Lanka SE Sweden	CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation			
	DE	Germany	u	Liechtenstein	SD	Sudan			
EE Estonia LR Liberia SG Singapore	DK	Denmark	LK	Sri Lanka	SE	Sweden			
	EE	Estonia	LR	Liberia	SG	Singapore			

PCT/US 99/18760

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C12N15/54 C12 C12N9/10 C12N5/10 C12N15/82 According to International Patent Classification (IPC) or to both national classification and IPC B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consumed during the international search (name of data base and, where practical, search terms used) STRAND, EPO-Internal, WPI Data, PAJ C. DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No WO 98 00549 A (WILLIAMSON RICHARD EDWARD 1-15 Х :PENG LIANGCAI (AU); ARIOLI ANTONIO (AU)) 8 January 1998 (1998-01-08) abstract page 4, line 10 - line 14 page 7, line 19 - line 29 page 8, line 16 - line 21 page 11, line 6 - line 12 page 17, line 4 - line 19 page 24, line 15 - line 18 page 28, line 15 - line 21 WO 98 18949 A (CALGENE INC ; PEAR JULIE R 1-15 Α (US); STALKER DAVID M (US); DELMER DEBOR) 7 May 1998 (1998-05-07) cited in the application abstract page 7, line 14 -page 9, line 25 -/--Х Patent family members are listed in annex. Further documents are listed in the commutation of box C. Special categories of cited documents: "T" later accument published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the *A* document defining the general state of the art which is not considered to be of particular relevance "E" earlier document but published on or after the international "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention citation or other special reason (as specified) cannot be considered to involve an inventive, step when the document is combined with one or more other such docu-"O" document referring to an oral disclosure, use, exhibition or ments, such combination being obvious to a person skilled other means document published prior to the international filing date but "&" document member of the same patent family later than the priority date claimed Date or making of the international search report Date of the actual completion of the international search 14 7.00 22 June 2000 Authorized officer Name and mailing address of the ISA European Patent Office, P.B. 5318 Patentiaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nt, Ceder, 0 Fax: (+31-70) 340-3016

1

1

7

International Application No
PCT/US 99/18760

cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002125688 HEIDELBERG DE Ac 048947 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002125689 HEIDELBERG DE AC AF027174 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140697 HEIDELBERG DE Ac 048948 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 20 January 1998 (1998-01-20), XP002140698 HEIDELBERG DE Ac AF030052 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140699 HEIDELBERG DE Ac 048946 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 June 1998 (1998-02-03), XP002140700 HEIDELBERG DE Ac AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE Ac AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, no. 5351,	99/18760
ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002125688 HEIDELBERG DE AC 048947 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002125689 HEIDELBERG DE AC AF027174 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140697 HEIDELBERG DE AC 048948 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 20 January 1998 (1998-01-20), XP002140698 HEIDELBERG DE AC AF030052 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140699 HEIDELBERG DE AC AF030052 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140699 HEIDELBERG DE AC 048946 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE AC AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE AC AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, no. 5351,	
cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002125688 HEIDELBERG DE Ac 048947 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002125689 HEIDELBERG DE AC AF027174 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140697 HEIDELBERG DE Ac 048948 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 20 January 1998 (1998-01-20), XP002140698 HEIDELBERG DE Ac AF030052 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140699 HEIDELBERG DE Ac 048946 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 June 1998 (1998-02-03), XP002140700 HEIDELBERG DE Ac AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE Ac AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, no. 5351,	Relevant to claim No.
the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002125689 HEIDELBERG DE AC AF027174 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140697 HEIDELBERG DE AC 048948 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 20 January 1998 (1998-01-20), XP002140698 HEIDELBERG DE AC AF030052 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140699 HEIDELBERG DE AC 048946 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE AC AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE AC AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, no. 5351,	15
-& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SCOUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140697 HEIDELBERG DE Ac 048948 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 20 January 1998 (1998-01-20), XP002140698 HEIDELBERG DE Ac AF030052 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140699 HEIDELBERG DE Ac 048946 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE Ac AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, no. 5351,	1
-& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 20 January 1998 (1998-01-20), XP002140698 HEIDELBERG DE Ac AF030052 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140699 HEIDELBERG DE Ac 048946 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE Ac AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, no. 5351,	15
-& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 1 June 1998 (1998-06-01), XP002140699 HEIDELBERG DE AC 048946 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE AC AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, no. 5351,	1
-& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" EMBL SEQUENCE DATABASE, 3 February 1998 (1998-02-03), XP002140700 HEIDELBERG DE Ac AF027173 the whole document -& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, no. 5351,	15
-& ARIOLI ET AL.: "Molecular analysis of cellulose biosynthesis in Arabidopsis" SCIENCE, vol. 279, no. 5351,	15
30 January 1998 (1998-01-30), pages	15
717-720, XP002124283 abstract; figure 3	1

Inten. .anal Application No PCT/US 99/18760

Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
WU ET AL.: "Cellulose synthase" EMBL SEQUENCE DATABASE, 1 August 1998 (1998-08-01), XP002140701 HEIDELBERG DE Ac 065338 the whole document	15
PEAR J R ET AL: "HIGHER PLANTS CONTAIN HOMOLOGS OF THE BACTERIAL CELA GENES ENCODINGTHE CATALYTIC SUBUNIT OF CELLULOSE SYNTHASE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, US, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 93, page 12637-12642 XP002061424 ISSN: 0027-8424	
AMOR Y ET AL: "EVIDENCE FOR A CYCLIC DIGUANYLIC ACID-DEPENDENT CELLULOSE SYNTHASE IN PLANTS" PLANT CELL,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 3, page 989-995 XP002061420 ISSN: 1040-4651	
US 5 723 764 A (SINGLÉTARY GEORGE WILLIAM ET AL) 3 March 1998 (1998-03-03) abstract; claims	2-12
WO 00 04166 A (THORPE ET AL.) 27 January 2000 (2000-01-27) abstract; claims	1-15
	EMBL SEQUENCE DATABASE, 1 August 1998 (1998-08-01), XP002140701 HEIDELBERG DE Ac 065338 the whole document PEAR J R ET AL: "HIGHER PLANTS CONTAIN HOMOLOGS OF THE BACTERIAL CELA GENES ENCODINGTHE CATALYTIC SUBUNIT OF CELLULOSE SYNTHASE" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, US, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, vol. 93, page 12637-12642 XP002061424 ISSN: 0027-8424 AMOR Y ET AL: "EVIDENCE FOR A CYCLIC DIGUANYLIC ACID-DEPENDENT CELLULOSE SYNTHASE IN PLANTS" PLANT CELL, US, AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 3, page 989-995 XP002061420 ISSN: 1040-4651 US 5 723 764 A (SINGLETARY GEORGE WILLIAM ET AL) 3 March 1998 (1998-03-03) abstract; claims WO 00 04166 A (THORPE ET AL.) 27 January 2000 (2000-01-27)

International application No. PCT/US 99/18760

INTERNATIONAL SEARCH REPORT

Boxi	Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)
This Inte	ernational Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1.	Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
2.	Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3.	Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II	Observations where unity of invention is lacking (Continuation of item 2 of first sheet)
This Inte	rnational Searching Authority found multiple inventions in this international application, as follows:
	see additional sheet
	As a result of the prior review under R. $40.2(e)$ PCT, no additional fees are to be refunded.
1.	As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.	As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. 🛛	As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
	1-15 all partly (inventions 1,2,3,4,5,7 and 15 searched)
4.	No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Remark o	The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: Invention 1: 1-15 all partial

An isolated nucleic acid selected from the groups as defined in claim 1 and uses of said nucleic acid, and a protein selected from the groups as defined in claim 15, where the nucleic acid sequence is SEQ ID NO 1 and the protein sequence is SEQ ID NO 2.

2. Claims: Inventions 2-15: Claims 1-15 all partial

Idem as subject 1 but limited to each of the nucleic acid sequences as in SEQ ID NOS 5, 9, 13, 17, 21, 25, 29, 33, 37, 41, 45, 49, 53, and 57 and corresponding protein sequences SEQ ID NOS 6, 10, 14, 18, 22, 26, 30, 34, 42, 46, 50, 54, and 58, where invention 2 is limited to SEQ ID NOS 5 and 6, invention 3 is limited to SEQ ID NOS 9 and 10,, invention 15 is limited to SEQ ID NOS 57 and 58.

Information on patent family members

PCT/US 99/18760

Patent document cited in search repor	t	Publication date		Patent family member(s)	Publication date	
WO 9800549	Α	08-01-1998	AU	3160397 A 2259126 A	21-01-1998	
			CA EP	0956353 A	08-01-1998 17-11-1999	
WO 9818949	Α	07-05-1998	AU	5092398 A	22-05-1998	
			BR	9712457 A	19-10-1999	
			EP	0938573 A	01-09-1999	
US 5723764	Α	03-03-1998	NONE			
WO 0004166	Α	27-01-2000	AU	5100199 A	07-02-2000	